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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/04, C12Q 1/68, A61K 48/00 — A1

(11) International Publication Number: **WO 00/20435**

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/23171

(22) International Filing Date: 5 October 1999 (05.10.99)

(30) Priority Data:
09/167,109 6 October 1998 (06.10.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/167,109 (CIP)
Filed on 6 October 1998 (06.10.98)

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ANTISENSE MODULATION OF EXPRESSION OF TUMOR NECROSIS FACTOR RECEPTOR–ASSOCIATED FACTORS (TRAFs)

(57) Abstract

Compositions and methods are provided for modulating the expression of tumor necrosis factor receptor–associated factor (TRAF). Antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding TRAF are preferred. Methods of using these compounds for modulation of TRAF expression and for treatment of diseases associated with expression of TRAF are provided.

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ANTISENSE MODULATION OF EXPRESSION OF TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTORS (TRAFs)

FIELD OF THE INVENTION

The present invention provides compositions and methods for modulating the expression of tumor necrosis factor receptor-associated factors (TRAFs). In particular, this invention relates to antisense compounds, particularly oligonucleotides, specifically hybridizable with nucleic acids encoding human TRAFs. Such oligonucleotides have been shown to modulate the expression of TRAFs.

BACKGROUND OF THE INVENTION

Tumor necrosis factor (TNF) receptor superfamily members regulate cellular proliferation, differentiation and apoptosis in inflammatory and immune responses. This receptor superfamily comprises a group of related cell-surface receptors including, but not limited to, types 1 and 2 TNF receptors (TNFR1 and TNFR2), Fas, CD27, 4-1BB, CD40 and CD30. Signaling through TNF receptor superfamily members is initiated by oligomerization of the receptors with trimeric ligands, bringing intracellular domains in close proximity (Pullen et al., *Biochemistry* **1998**, 37, 11836-11845). Two families of adaptor proteins that associate with TNF receptor superfamily members have been identified: the TNF receptor-associated factor (TRAF) family, and the death domain-containing protein family.

Members of the TRAF family of proteins share an amino-terminal RING finger motif and a homologous carboxy-terminal region, referred to in the art as the TRAF domain (Yuan, J., *Curr. Opin. Cell Biol.* **1997**, 9, 247-251. This conserved carboxy-terminal region binds to receptor cytoplasmic domains and mediates interactions with the signaling proteins NF-κB inducing kinase (NIK) and I-TRAFT/TANK (Cheng et al., *Science* **1995**, 267, 1494-1498;

Cheng, G. and Baltimore, D., *Genes Dev.* **1996**, 10, 963-973; Rothe et al., *Proc. Natl Acad. Sci. USA* **1996**, 93, 8241-8246; Malinin et al., *Nature* **1997**, 385, 540-544). A predicted coiled-coil region mediating TRAF homo- and hetero-oligomerization is in a less conserved region N-terminal to the TRAF domain (Cao et al., *Nature* **1996**, 383, 443-446; Cheng et al., *Science* **1995**, 267, 1494-1498; Rothe et al., *Cell* **1994**, 78, 681-692; Sato et al., *FEBS Lett* **1995**, 358, 113-118; and Takeuchi et al., *J. Biol. Chem* **1996**, 271, 19935-19942).

The mammalian TRAF family currently includes six members, TRAF-1, TRAF-2, TRAF-3, TRAF-4, TRAF-5 and TRAF-6. These proteins have generally been found within the cytosols of cells, either in association with cytosolic vesicles or at the plasma membrane after addition of selected TNF family cytokines to the cells. Members of the TRAF family mediate signals for various different receptors. Subsets of TRAF family members have been shown to interact with the TNF receptor family members (TNFR2, CD40, CD30, LTβR, ATAR, OX40 and 4-1BB).

For example, TRAF-1 and TRAF-2 were identified by their ability to interact with the cytoplasmic domains of TNFR2 (Rothe et al., *Cell* **1994**, 78, 681-691). TNFR2 has been associated with TNF's ability to stimulate cell proliferation and activation of NFκB (Tartaglia et al., *Proc. Natl Acad. Sci. USA* **1991**, 88, 9292-9296). TRAF-1 is believed to be involved in the regulation of apoptosis (Speiser et al., *J. Exp. Med.* **1997**, 185, 1777-1783). Depletion of TRAF-2 and its co-associated proteins has also been shown to increase the sensitivity of the cell to undergo apoptosis during activation of death inducing receptors such as TNFR1 (Duckett, C.S. and Thompson, C.B., *Genes & Development* **1997**, 11, 2810-2821; Yeh et al., *Immunity* **1997**, 7, 715-725). Accordingly, the rate of

receptor-mediated TRAF-2 consumption and TRAF-2 translation has been suggested to play a dynamic role in the regulation of cell survival (Duckett, C.S. and Thompson, C.B., *Genes & Development* **1997**, 11, 2810-2821). Targeted disruption of the TRAF-2 gene in mice has also been shown to generate severe defects in c-Jun N-terminal kinase (JNK) activation through TNFR1 (Yeh et al., *Immunity* **1997**, 7, 715-725).

TRAF-2 (Rothe et al., *Science* **1995**, 269, 1424-1427), TRAF-3 (Cheng et al., *Science* **1995**, 267, 1494-1498), TRAF-5 (Ishida et al., *Proc. Natl Acad. Sci USA* **1996**, 93, 9437-9442) and TRAF-6 (Pullen et al., *Biochemistry* **1998**, 37, 11836-11845) have also been shown to interact with the B lymphocyte receptor CD40. CD40 is a TNF receptor superfamily member that provides activation signals in antigen presenting cells such as B cells, macrophages and dendritic cells. Activation of CD40 leads to B-cell survival, growth and differentiation. In 293T cells, expression of TRAF-3 suppressed constitutive activity of NFκB, whereas expression of TRAF-5 induced NFκB activity. Targeted disruption of the TRAF-3 gene in mice causes impaired immune responses to T-dependent antigens and results in early postnatal lethality (Xu et al., *Immunity* **1996**, 5, 407-415). TRAF-2, TRAF-5 or TRAF-6 overexpression in mammalian cells also induces JNK activation.

TRAF-4 is expressed in breast cancers. In *in vitro* binding assays, TRAF-4 has been shown to interact with the cytosolic domain of the lymphotoxin-β receptor (LTβR) and weakly with the p75 nerve growth factor receptor but not with TNFR1, TNFR2, Fas or CD40 (Karjewska et al., *Am. J. of Pathol.* **1998**, 152, 6, 1549-1561).

TRAF-6 has also been reported to mediate the signal transduction pathway induced by IL-1 to activate NFκB by recruiting IL-1 receptor associated kinase (IRAK), a serine/threonine kinase (Cao et al., *Nature* **1996** 93:9437-

9442). Thus, the role of TRAFs extends beyond being signal transducers for the TNF-receptor superfamily.

The TRAF-5 protein and DNA encoding TRAF-5 are disclosed in WO97/38099. Also disclosed in WO97/38099 is an antisense oligonucleotide against the DNA, an anti-TRAF-5 antibody, a vector containing the DNA, transformants containing this vector and methods of producing TRAF-5 with this vector. In addition, this PCT application discloses methods of screening substances binding to TRAF-5 and substances regulating the activity and expression of this protein.

A TRAF family molecule, a polynucleotide coding for this molecule, an antibody against the molecule and an antisense polynucleotide of the molecule are also disclosed in WO97/31110. Disclosed in this PCT application are the base sequence of the gene and the amino acid of this "unknown" TRAF family molecule, which in addition to the antibody, are suggested to provide means for elucidating the functions of the proteins and the signal transducer system of a TNF-R family in which this molecule participates, to provide probes for research and diagnosis, and to indicate applications for therapeutic agents.

Currently, however, there are no known therapeutic agents which effectively inhibit the synthesis of one or more selected TRAF family members. Consequently, there is a long-felt need for agents capable of effectively inhibiting TRAF expression. Antisense oligonucleotides against one or more TRAFs may therefore prove to be uniquely useful in a number of therapeutic, diagnostic and research applications.

SUMMARY OF THE INVENTION

The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to a nucleic acid encoding a selected tumor necrosis factor receptor-associated factor (TRAF), and

which modulate the expression of the selected TRAF. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating the expression of TRAFs in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of a selected TRAF by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding selected tumor necrosis factor receptor-associated factors (TRAFs), ultimately modulating the amount of the selected TRAF produced. This is accomplished by providing antisense compounds which specifically hybridize with one or more nucleic acids encoding the selected TRAF. By "selected TRAF" it is meant any member of the TRAF family of proteins, most preferably TRAF-1, TRAF-2, TRAF-3, TRAF-4, TRAF-5 or TRAF-6. As used herein, the terms "target nucleic acid" and "nucleic acid encoding TRAF" encompass DNA encoding a TRAF family member, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of

RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of the selected TRAF. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target.

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multi-step process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is one or more nucleic acid molecules encoding one or more selected TRAFs. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation

codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function *in vivo*. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA molecule transcribed from a gene encoding a TRAF, regardless of the sequence(s) of such codons.

It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, *i.e.*, 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the

translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns", which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites, *i.e.*, intron-exon junctions, may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

Once one or more target sites have been identified,

oligonucleotides are chosen which are sufficiently complementary to the target, *i.e.*, hybridize sufficiently well and with sufficient specificity, to give the desired effect.

In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, *i.e.*,

under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed.

Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimens in cells, tissues and animals, especially humans.

In the context of this invention, the term "oligonucleotide refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

While antisense oligonucleotides are a preferred form of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30 nucleobases. Particularly preferred are antisense oligonucleotides comprising from about 8 to about 30 nucleobases (*i.e.*, from about 8 to about 30 linked nucleosides). As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the

backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside);

siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching

of PNA compounds can be found in Nielsen et al., *Science*, **1991**, 254, 1497-1500.

Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above referenced U.S. Patent 5,489,677, and the amide backbones of the above referenced U.S. Patent 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Patent 5,034,506.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also

known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, **1995**, 78, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in United States patent application Serial Number 09/016,520, filed on January 30, 1998, which is commonly owned with the instant application and the contents of which are herein incorporated by reference.

Other preferred modifications include 2'-methoxy (2'-O-CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotides. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is incorporated herein by reference.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-Me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives

of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Patent 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, *J.I., ed.* John Wiley & Sons, **1990**, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, **1991**, *30*, 613, and those disclosed by Sanghvi, Y.S., Crooke, S.T. and Lebleu, B. eds., *Antisense Research and Applications*, CRC Press, Boca Raton, **1993**, pp. 289-302. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, **1993**, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, U.S.: 3,687,808; 4,845,205;

5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,681,941; and 5,750,692, each of which is herein incorporated by reference.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, **1989**, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.*, **1994**, 4, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, **1992**, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, **1993**, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, **1992**, 20, 533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, **1991**, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, **1990**, 259, 327-330; Svinarchuk et al., *Biochimie*, **1993**, 75, 49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, **1995**, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, **1990**, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, **1995**, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, **1995**, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, **1995**, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, **1996**, 277, 923-937.

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit *i.e.*, a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is

a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

The antisense compounds of the invention are synthesized *in vitro* and do not include antisense

compositions of biological origin, or genetic vector constructs designed to direct the *in vivo* synthesis of antisense molecules.

The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S.: 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (*i.e.*, drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE

[(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 or in WO 94/26764.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: *i.e.*, salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.*, **1977**, 66, 1-19).

For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, *etc.*; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

The antisense compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of one or more members of the TRAF family is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical compositions by adding an

effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, *e.g.*, to prevent or delay infection, inflammation or tumor formation, for example.

The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding one or more selected TRAFs, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridization of the antisense oligonucleotides of the invention with a nucleic acid encoding one or more TRAFs can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of TRAF in a sample may also be prepared.

The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; (intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

For example, pharmaceutical compositions and/or formulations comprising the oligonucleotides of the present invention may include penetration enhancers in order to enhance the alimentary delivery of the oligonucleotides. Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, fatty acids, bile salts, chelating agents, surfactants and non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, 8, 91-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, 7, 1-33). One or more penetration enhancers from one or more of these broad categories may be included.

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, recinleate, monoolein (a.k.a. 1-monooleoyl-rac-

glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, mono- and di-glycerides and physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, **1991**, 8, 2, 91-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, **1990**, 7, 1, 1-33; El-Hariri et al., *J. Pharm. Pharmacol.*, **1992**, 44, 651-654). Examples of some presently preferred fatty acids are sodium caprate and sodium laurate, used singly or in combination at concentrations of 0.5 to 5%.

The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al., eds., McGraw-Hill, New York, NY, **1996**, pages 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives.

Regardless of the method by which the antisense compounds of the invention are introduced into a patient, colloidal dispersion systems may be used as delivery vehicles to enhance the *in vivo* stability of the compounds and/or to target the compounds to a particular organ, tissue or cell type. Colloidal dispersion systems include, but are not limited to, macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, liposomes and lipid:oligonucleotide complexes of uncharacterized structure. A preferred colloidal

dispersion system is a plurality of liposomes. Liposomes are microscopic spheres having an aqueous core surrounded by one or more outer layer(s) made up of lipids arranged in a bilayer configuration (see, generally, Chonn et al., *Current Op. Biotech.*, **1995**, 6, 698-708).

Liposome preparation is described in pending United States patent application 08/961,469, filed on October 31, 1997, which is commonly owned with the instant application and which is herein incorporated by reference.

Certain embodiments of the invention provide for liposomes and other compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Two or more combined compounds may be used together or sequentially.

The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be

effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily, to once every 20 years.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

Nucleoside Phosphoramidites for Oligonucleotide Synthesis

Deoxy and 2'-alkoxy amidites

2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites are purchased from commercial sources (*e.g.*, Chemgenes, Needham, MA or Glen Research, Inc. Sterling VA). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Patent 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides is utilized, except the wait step after pulse delivery of tetrazole and base is increased to 360 seconds.

Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides were synthesized according to

published methods (Sanghvi, et. al., *Nucleic Acids Research*, **1993**, 21, 3197-3203] using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham, MA).

2'-Fluoro amidites

2'-Fluorodeoxyadenosine amidites

2'-fluoro oligonucleotides are synthesized as described previously by Kawasaki, et. al., *J. Med. Chem.*, **1993**, 36, 831-841 and U.S. Patent 5,670,633, herein incorporated by reference. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine are synthesized utilizing commercially available 9-beta-D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom is introduced by a S_N2-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine is selectively protected in moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups is accomplished using standard methodologies and standard methods are used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

The synthesis of 2'-deoxy-2'-fluoroguanosine is accomplished using tetraisopropyldisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-arabinofuranosylguanosine. Deprotection of the TPDS group is followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation is followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies are used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

Synthesis of 2'-deoxy-2'-fluorouridine is accomplished by the modification of a literature procedure in which 2,2'-anhydro-1-beta-D-arabinofuranosyluracil is treated with 70% hydrogen fluoride-pyridine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

2'-Fluorodeoxycytidine

2'-deoxy-2'-fluorocytidine is synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N4-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

2'-O-(2-Methoxyethyl) modified amidites

2'-O-Methoxyethyl-substituted nucleoside amidites were prepared as follows, or alternatively, as per the methods of Martin, P., *Helvetica Chimica Acta*, **1995**, 78, 486-504.

2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-methyluridine]

5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum dried in a vacuum oven (60°C at 1 mm Hg for 24 hours) to

give a solid that was crushed to a light tan powder (57 g, 85% crude yield). The NMR spectrum was consistent with the structure, contaminated with phenol as its sodium salt (ca. 5%). The material was used as is for further reactions (or purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid, mp 222-4°C).

2'-O-Methoxyethyl-5-methyluridine

2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating for 48 hours at 155-160°C, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH_3CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH_2Cl_2/Acetone/MeOH (20:5:3) containing 0.5% Et_3NH. The residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with

CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L) and extracted with 2x500 mL of saturated $NaHCO_3$ and 2x500 mL of saturated NaCl. The organic phase was dried over Na_2SO_4, filtered and evaporated. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/Hexane/Acetone (5:5:1) containing 0.5% Et_3NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by tlc by first quenching the tlc sample with the addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in $CHCl_3$ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water layers were back extracted with 200 mL of $CHCl_3$. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane (4:1). Pure product fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a

solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to -5°C and stirred for 0.5 hours using an overhead stirrer. $POCl_3$ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the latter solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids removed by filtration. The filtrate was washed with 1x300 mL of $NaHCO_3$ and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2x200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas was added and the vessel heated to 100°C for 2 hours (tlc showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent evaporated to give 85 g (95%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyl-cytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) is added with stirring.

The mixture was stirred for 3 hours (tlc showing the reaction to be approximately 95% complete). The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was then dissolved in $CHCl_3$ (700 mL) and extracted with saturated $NaHCO_3$ (2x300 mL) and saturated NaCl (2x300 mL), dried over $MgSO_4$ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (tlc showing the reaction to be 95% complete). The reaction mixture was extracted with saturated $NaHCO_3$ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over $MgSO_4$ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc/Hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-(Aminooxyethyl) nucleoside amidites and 2'-(dimethylaminooxyethyl) nucleoside amidites

Aminooxyethyl and dimethylaminooxyethyl amidites are prepared as per the methods described in United States patent applications serial number 10/037,143, filed February 14, 1998, and serial number 09/016,520, filed January 30, 1998, each of which is herein incorporated by reference.

Example 2

Oligonucleotide synthesis

Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) were synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 seconds and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 hours), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution.

Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, herein incorporated by reference.

Example 3

Oligonucleoside Synthesis

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

Example 4

PNA Synthesis

Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, **1996**, 4, 5-23. They may also be prepared in accordance

with U.S. Patents 5,539,082, 5,700,922, and 5,719,262, herein incorporated by reference.

Example 5

Synthesis of Chimeric Oligonucleotides

Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3' "wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers".

[2'-O-Me]--[2'-deoxy]--[2'-O-Me] Chimeric Phosphorothioate Oligonucleotides

Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by increasing the wait step after the delivery of tetrazole and base to 600 seconds repeated four times for RNA and twice for 2'-O-methyl. The fully protected oligonucleotide is cleaved from the support and the phosphate group is deprotected in 3:1 Ammonia/Ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hours at room temperature is then done to deprotect all bases and sample was again lyophilized to dryness. The pellet is resuspended in 1M TBAF in THF for 24 hours at

room temperature to deprotect the 2' positions. The reaction is then quenched with 1M TEAA and the sample is then reduced to 1/2 volume by rotovac before being desalted on a G25 size exclusion column. The oligo recovered is then analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

[2'-O-(2-Methoxyethyl)]--[2'-deoxy]--[2'-O-(Methoxyethyl)] Chimeric Phosphorothioate Oligonucleotides

[2'-O-(2-methoxyethyl)]--[2'-deoxy]--[-2'-O-(methoxyethyl)] chimeric phospnorothioate oligonucleotides were prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

[2'-O-(2-Methoxyethyl)Phosphodiester]--[2'-deoxy Phosphorothioate]--[2'-O-(2-Methoxyethyl) Phosphodiester] Chimeric Oligonucleotides

[2'-O-(2-methoxyethyl phosphodiester]--[2'-deoxy phosphorothioate]--[2'-O-(methoxyethyl) phosphodiester] chimeric oligonucleotides were prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidization with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Deaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to United States patent 5,623,065, herein incorporated by reference.

Example 6

Oligonucleotide Isolation

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides or oligonucleosides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Synthesized oligonucleotides were analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis were periodically checked by ^{31}P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides are purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* **1991**, 266, 18162-18171. Results obtained with HPLC-purified material are similar to those obtained with non-HPLC purified material.

Example 7

Oligonucleotide Synthesis - 96 Well Plate Format

Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a standard 96 well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial vendors (*e.g.* PE-Applied Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ). Non-standard nucleosides were synthesized as per known literature or patented methods. They were utilized as base protected beta-cyanoethyldiisopropyl phosphoramidites.

Oligonucleotides were cleaved from support and deprotected with concentrated NH_4OH at elevated temperature (55-60°C) for 12-16 hours and the released product then dried *in vacuo*. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples were then diluted utilizing robotic pipettors.

Example 8

Oligonucleotide Analysis - 96 Well Plate Format

The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96 well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (*e.g.*, Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

Example 9

Cell culture and oligonucleotide treatment

The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR, RNAse protection assay (RPA) or Northern blot analysis. The following four cell types are provided for illustrative purposes, but other cell types can be routinely used.

T-24 cells:

The transitional cell bladder carcinoma cell line T-24 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). T-24 cells are routinely cultured in complete McCoy's 5A basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells are seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

A549 cells:

The human lung carcinoma cell line A549 is obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells are routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells are routinely passaged by trypsinization and dilution when they reach 90% confluence.

NHDF cells:

Human neonatal dermal fibroblast (NHDF) are obtained from the Clonetics Corporation (Walkersville MD). NHDFs are routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville, MD) supplemented as

recommended by the supplier. Cells are maintained for up to 10 passages as recommended by the supplier.

HEK cells:

Human embryonic keratinocytes (HEK) are obtained from the Clonetics Corporation (Walkersville, MD). HEKs are routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville, MD) formulated as recommended by the supplier. Cells are routinely maintained for up to 10 passages as recommended by the supplier.

Treatment with antisense compounds:

When cells reach 80% confluency, they are treated with oligonucleotide. For cells grown in 96-well plates, wells are washed once with 200 μL OPTI-MEM™-1 reduced-serum medium (Gibco BRL) and then treated with 130 μL of OPTI-MEM™-1 containing 3.75 μg/mL LIPOFECTIN™ (Gibco BRL) and the desired oligonucleotide at a final concentration of 150 nM. After 4 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16 hours after oligonucleotide treatment.

Example 10

Analysis of oligonucleotide inhibition of TRAF expression

Antisense modulation of TRAF expression can be assayed in a variety of ways known in the art. For example, TRAF mRNA levels can be quantitated by, *e.g.*, Northern blot analysis, RNAse protection assay (RPA), competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are taught in, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 1, John Wiley & Sons, Inc., **1993**, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3. Northern blot analysis is routine in the art and is taught in, for example, Ausubel,

et al., *Current Protocols in Molecular Biology*, Volume 1, John Wiley & Sons, Inc., **1996**, pp. 4.2.1-4.2.9. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions. Other methods of PCR are also known in the art.

TRAF protein levels can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Antibodies directed to TRAF can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional antibody generation methods. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 2, John Wiley & Sons, Inc., **1997**, pp. 11.12.1-11.12.9. Preparation of monoclonal antibodies is taught in, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 2, John Wiley & Sons, Inc., **1997**, pp. 11.4.1-11.11.5.

Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 2, John Wiley & Sons, Inc., **1998**, pp. 11.4.1-11.11.5. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 2, John Wiley & Sons, Inc., **1997**, pp. 10.8.1-10.8.21. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, et al., *Current Protocols in*

Molecular Biology, Volume 2, John Wiley & Sons, Inc., 1991, pp. 11.2.1-11.2.22.

Example 11

Poly(A)+ mRNA isolation

Poly(A)+ mRNA is isolated according to Miura et al., *Clin. Chem.*, 1996, 42, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, et al., *Current Protocols in Molecular Biology*, Volume 1, John Wiley & Sons, Inc., 1993, pp. 4.5.1-4.5.3. Briefly, for cells grown on 96-well plates, growth medium is removed from the cells and each well is washed with 200 μL cold PBS. 60 μL lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) is added to each well, the plate is gently agitated and then incubated at room temperature for 5 minutes. 55 μL of lysate is transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine, CA). Plates are incubated for 60 minutes at room temperature, washed 3 times with 200 μL of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate is blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 μL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C is added to each well, the plate is incubated on a 90°C hot plate for 5 minutes, and the eluate is then transferred to a fresh 96-well plate.

Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Example 12

Total RNA Isolation

Total mRNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia, CA) following the manufacturer's recommended procedures. Briefly, for

cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μL cold PBS. 100 μL Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 100 μL of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 15 seconds. 1 mL of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 10 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 60 μL water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step was repeated with an additional 60 μL water.

Example 13

Real-time Quantitative PCR Analysis of TRAF mRNA Levels

Quantitation of TRAF mRNA levels is determined by real-time quantitative PCR using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR

reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (*e.g.*, JOE or FAM, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 5' end of the probe and a quencher dye (*e.g.*, TAMRA, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

PCR reagents are obtained from PE-Applied Biosystems, Foster City, CA. RT-PCR reactions are carried out by adding 25 μL PCR cocktail (1x TAQMAN™ buffer A, 5.5 mM $MgCl_2$, 300 μM each of dATP, dCTP and dGTP, 600 μM of dUTP, 100 nM each of forward primer, reverse primer, and probe, 20 Units RNAse inhibitor, 1.25 Units AMPLITAQ GOLD™, and 12.5 Units MuLV reverse transcriptase) to 96 well plates containing 25 μL poly(A) mRNA solution. The RT reaction is

carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol are carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension). TRAF probes and primers are designed to hybridize to the human TRAF sequence, using published sequence information. For example, GenBank Accession No. U19261, Locus name "HSU 19261" SEQ ID NO: 1; GenBank Accession No. U12597, Locus name "HSU12597" SEQ ID NO. 2; GenBank Accession No U21092, Locus name "HSU21092 SEQ ID NO: 3; GenBank Accession No. X80200, Locus name "HSMLN62" SEQ ID NO. 4; GenBank Accession No. AB000509, Locus name "AB000509 SEQ ID NO. 5; GenBank Accession No. U78798, Locus name "HSU78798" SEQ ID NO. 6.

Example 14

Antisense inhibition of TRAF-1 expression- phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target different regions of the human TRAF-1 RNA, using published sequences (GenBank accession number U19261, incorporated herein as SEQ ID NO: 1). The oligonucleotides are shown in Table 1. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. U19261), to which the oligonucleotide binds. All compounds in Table 1 are oligodeoxynucleotides with phosphorothioate backbones (internucleoside linkages) throughout.

TABLE 1

Nucleotide Sequences of Human TRAF-1 Phosphorothioate Oligonucleotides

	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26698	TTTAAGTTGCTCCAGGGC	7	0028-0045	5'-UTR
26699	GCCGGGCGAGGACTGCTG	8	0093-0110	coding
26700	GCAGACGGTGGGAGGGCA	9	0139-0156	coding
26701	CTGGGCTCCTTTGGGTCC	10	0159-0176	coding
26702	CACAGCAGAGAGCCCTGG	11	0173-0190	coding
26703	ATTCCTCGGGTTCTCAGA	12	0202-0219	coding
26704	CCATTCCTCGGGTTCTCA	13	0204-0221	coding
26705	CCTCGCCATTCCTCGGGT	14	0209-0226	coding
26706	GATCCTCGCCATTCCTCG	15	0212-0229	coding
26707	AGACGGCTTCCTGGGCTT	16	0270-0287	coding
26708	TTGAAGGAGCAGCCGACA	17	0351-0368	coding
26709	GGCCTTCCACTGTTTCAT	18	0442-0459	coding
26710	CCACTTCCACGGCTGCCT	19	0527-0544	coding
26711	CGCCTGGTGACATTGGTG	20	0894-0911	coding
26712	CGCATCATACTCCCCTCT	21	1063-1080	coding
26713	AGGCGTCAATGGCGTGCT	22	1142-1159	coding
26714	GGAAGGCGTCAATGGCG1	23	1145-1162	coding
26715	GGAAGAAGAGTGGGCATC	24	1223-1240	coding
26716	CGTAGGCGTGCTTGGGTG	25	1259-1276	coding
26717	GCCCCGCCCACCCTAAGT	26	1321-1338	stop
26718	GGAGCCCCGCCCACCCTA	27	1324-1341	stop
26719	CTCAGGAGCCCCGCCCAC	28	1328-1345	3'-UTR
26720	AAGGGCAGGGCATCACAG	29	1380-1397	3'-UTR
26721	TTTGTGCCCTGAGGTCTT	30	1405-1422	3'-UTR

	26722	CACCCATCTTTGTGCCCT	31	1413-1430	3'-UTR
	26723	GGCCTCCCAGTGTCGCAT	32	1570-1587	3'-UTR
	26724	CCCGGTCCTGTTTCTGAC	33	1756-1773	3'-UTR
	26725	GCACCCCATCCCTTCCAC	34	1773-1790	3'-UTR
5	26726	TGGAGCCGTCTGGGTTTG	35	1837-1854	3'-UTR
	26727	GTCTTCAAATCCAACCCC	36	1871-1888	3'-UTR
	26728	TTCTGGGCTGGAAGGAAA	37	1896-1913	3'-UTR
	26729	ACTTTCTGGGCTGGAAGG	38	1899-1916	3'-UTR
	26730	AGAGACTTTCTGGGCTGG	39	1903-1920	3'-UTR
10	26731	TTTCCAGAACCCCTGTAG	40	1955-1972	3'-UTR
	26732	ATGTTTCCAGAACCCCTG	41	1958-1975	3'-UTR
	26733	GGGCTGGGTGTGCTCCTG	42	2090-2107	3'-UTR
	26734	TTTATGCCCCTCTTCTTC	43	2204-2221	3'-UTR
	26735	GGAAAGTTTATGCCCCTC	44	2210-2227	3'-UTR
15	26736	TACGGGATTCTGGAAAGC	45	2257-2274	3'-UTR
	26737	AGGTGTTACGGGATTCTG	46	2263-2280	3'-UTR

[1] All cytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

20

[2] Coordinates from GenBank Accession No. U19261, locus name "HSU19261" SEQ ID NO.1.

Example 15:

25 **Antisense inhibition of TRAF-1 expression- phosphorothioate 2'-MOE gapmer oligonucleotides**

In accordance with the present invention, a second series of oligonucleotides targeted to human TRAF-1 were synthesized. The oligonucleotide sequences are shown in

30 Table 2. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. U19261), to which the oligonucleotide binds.

All compounds in Table 2 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines.

TABLE 2

Nucleotide Sequences of Human TRAF-1 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26738	TTTAAGTTGCTCCAGGGC	7	0028-0045	5'-UTR
26739	GCCGGGCGAGGACTGCTG	8	0093-0110	coding
26740	GCAGACGGTGGGAGGGCA	9	0139-0156	coding
26741	CTGGGCTCCTTTGGGTCC	10	0159-0176	coding
26742	CACAGCAGAGAGCCCTGG	11	0173-0190	coding
26743	ATTCCTCGGGTTCTCAGA	12	0202-0219	coding
26744	CCATTCCTCGGGTTCTCA	13	0204-0221	coding
26745	CCTCGCCATTCCTCGGGT	14	0209-0226	coding
26746	GATCCTCGCCATTCCTCG	15	0212-0229	coding
26747	AGACGGCTTCCTGGGCTT	16	0270-0287	coding
26748	TTGAAGGAGCAGCCGACA	17	0351-0368	coding
26749	GGCCTTCCACTGTTTCAT	18	0442-0459	coding
26750	CCACTTCCACGGCTGCCT	19	0527-0544	coding
26751	CGCCTGGTGACATTGGTG	20	0894-0911	coding
26752	CGCATCATACTCCCCTCT	21	1063-1080	coding
26753	AGGCGTCAATGGCGTGCT	22	1142-1159	coding

26754	GGAAGGCGTCAATGGCGT	23	1145-1162	coding
26755	GGAAGAAGAGTGGGCATC	24	1223-1240	coding
26756	CGTAGGCGTGCTTGGGTG	25	1259-1276	coding
26757	GCCCCGCCCACCCTAAGT	26	1321-1338	stop
26758	GGAGCCCCGCCCACCCTA	27	1324-1341	stop
26759	CTCAGGAGCCCCGCCCAC	28	1328-1345	3'-UTR
26760	AAGGGCAGGGCATCACAG	29	1380-1397	3'-UTR
26761	TTTGTGCCCTGAGGTCTT	30	1405-1422	3'-UTR
26762	CACCCATCTTTGTGCCCT	31	1413-1430	3'-UTR
26763	GGCCTCCCAGTGTCGCAT	32	1570-1587	3'-UTR
26764	CCCGGTCCTGTTTCTGAC	33	1756-1773	3'-UTR
26765	GCACCCCATCCCTTCCAC	34	1773-1790	3'-UTR
26766	TGGAGCCGTCTGGGTTTG	35	1837-1854	3'-UTR
26767	GTCTTCAAATCCAACCCC	36	1871-1888	3'-UTR
26768	TTCTGGGCTGGAAGGAAA	37	1896-1913	3'-UTR
26769	ACTTTCTGGGCTGGAAGG	38	1899-1916	3'-UTR
26770	AGAGACTTTCTGGGCTGG	39	1903-1920	3'-UTR
26771	TTTCCAGAACCCCTGTAG	40	1955-1972	3'-UTR
26772	ATGTTTCCAGAACCCCTG	41	1958-1975	3'-UTR
26773	GGGCTGGGTGTGCTCCTG	42	2090-2107	3'-UTR
26774	TTTATGCCCCTCTTCTTC	43	2204-2221	3'-UTR
26775	GGAAAGTTTATGCCCCTC	44	2210-2227	3'-UTR
26776	TACGGGATTCTGGAAAGC	45	2257-2274	3'-UTR
26777	AGGTGTTACGGGATTCTG	46	2263-2280	3'-UTR

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No. 19261, locus name "HSU19261" SEQ ID NO. 1.

Example 16:

Antisense inhibition of TRAF-2 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a series of oligonucleotides targeted to human TRAF-2 were synthesized. The oligonucleotide sequences are shown in Table 3. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. U12597), to which the oligonucleotide binds.

All compounds in Table 3 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of eight 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by six-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) in the central "deoxy gap" and phosphodiester (P=O) in the wings. Cytidine residues in the 2'-MOE wings are 5-methylcytidines.

TABLE 3:

Nucleotide Sequences of TRAF-2 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
16827	GoToCoGoCoAsGsCsGsCsGsCsCsGsGoAoAoToToC	47	0001-0020	5'-UTR
16828	CoCoAoAoCoGsGsTsCsGsCsAsGsCsGoCoGoCoCoG	48	0007-0026	5'-UTR
16829	CoAoGoCoCoAsTsGsAsGsAsGsCsTsGoToGoAoCoC	49	0042-0061	AUG
16830	AoCoGoCoToAsGsCsTsGsCsAsGsCsCoAoToGoAoG	50	0052-0071	AUG
16831	GoCoCoAoCoAsCsTsGsCsGsCsCsTsGoGoAoAoGoG	51	0185-0204	coding
16832	CoCoGoGoCoAsGsGsCsTsCsTsCsCsAoCoCoToCoC	52	0348-0367	coding
16833	GoCoAoGoCoGsGsCsCsTsTsCsGsTsGoGoCoAoGoC	53	0422-0441	coding
16834	CoCoToCoGoTsGsGsTsGsCsGsCsCsToToCoAoCoG	54	0576-0595	coding
16835	CoToCoGoAoCsAsCsTsTsGsCsCsAsCoAoAoGoToC	55	0675-0694	coding
16836	CoAoCoToGoCsAsCsCsTsCsGsTsGsCoToCoCoToG	56	0751-0770	coding
16837	CoCoToCoToGsCsAsGsGsAsGsCsTsCoToGoAoCoC	57	0848-0867	coding
16838	CoAoGoCoCoGsGsTsGsCsTsGsCsCsGoGoCoToGoC	58	0962-0981	coding
16839	CoCoGoGoToGsCsCsGsTsCsGsCsCsGoToToCoAoG	59	1240-1259	coding
16840	AoCoGoToCoGsGsGsCsCsTsGsAsAsGoGoCoGoToC	60	1387-1406	coding

16841	CoToGoToCoAsGsGsTsCsCsAsCsAsAoToGoGoCoC	61	1533-1552	coding
16842	GoCoCoGoGoCsTsGsTsGsCsCsTsGsGoCoToGoCoC	62	1590-1609	3'-UTR
16843	CoToToGoGoCsTsGsCsAsGsGsCsCsGoAoCoAoCoC	63	1685-1704	3'-UTR
16844	CoGoGoCoCoAsAsTsGsCsCsAsCsCsAoCoAoGoCoC	64	1789-1808	3'-UTR
16845	AoCoToGoToGsCsTsCsCsTsGsCsTsAoCoAoToGoG	65	1916-1935	3'-UTR
16846	GoCoToCoToGsGsCsCsAsGsCsAsGsGoAoGoGoCoC	66	1994-2013	3'-UTR
16847	CoCoAoCoAoGsCsCsAsGsCsCsTsGsGoCoCoCoAoAoG	67	2117-2136	3'-UTR
16848	CoToCoToGoTsCsTsTsCsGsTsGsAsGoCoToGoGoA	68	2221-2240	3'-UTR
26264	CoCoToCoGoTsGsCsTsGsCsGsGsCsToToCoAoCoG	69	mismatch	
26266	CoCoToGoGoTsGsCsTsCsCsGsGsCsToToCoAoCoG	70	mismatch	
27693	CoCoToCoGsTsGsGsTsGsCsGsCsCsTsToCoAoCoG	54	0576-0595	coding
27694	CsCsTsCsGsTsGsGsTsGsCsGsCsCsTsTsCsAsCsG	54	0576-0595	coding

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy). All 2'-methoxyethoxy cytidines are 5-methyl-cytidines, underlined "C" residues are 5-methyl-cytidines; "s" linkages are phosphorothioate linkages, "o" linkages are phosphodiester linkages.

[2] Coordinates from GenBank Accession No. U12597, locus name "HSU12597" SEQ ID NO.2.

HMVEC (human dermal microvascular) cells were purchased from Clonetics (San Diego CA) and cultivated in endothelial basal medium (EBM) supplemented with 10% fetal bovine serum (HyClone, Logan UT). Cells were grown in 100 mm petri dishes until 70-80% confluent and then treated with oligonucleotide in the presence of cationic lipid. Briefly, cells were washed with PBS and OPTI-MEM®. OPTI-MEM® containing 10 μg/mL LIPOFECTIN® was added to the cells, followed by addition of oligonucleotide. The cells were incubated for 3-4 hours at 37° C, washed once with EBM/1% FBS, and allowed to recover. For determination of mRNA levels by Northern blot, total RNA was prepared from cells by the guanidinium isothiocyanate procedure or by the Qiagen RNEASY™ method (Qiagen, Valencia, CA). Northern blot analysis was performed by standard methods (for example, Ausubel, et al. *Current Protocols in Molecular Biology*, Vol. 1, John Wiley and Sons, Inc., **1996**, pp.4.2.1-4.2.9). The probe was a PCR-labeled 1-kb fragment of TRAF-2 amplified by RT-PCR according to the method of Bednarczuk et al., *Biotechniques*, **1991**, 10,478. RNA was quantified and normalized to G3PDH mRNA levels using a Molecular Dynamics (Sunnyvale, CA) PhosphorImager in accordance with manufacturer's instructions.

Results are shown in Table 4. Reduction of TRAF-2 mRNA levels with oligonucleotide 16834 (SEQ ID NO. 54) was determined to be dose-dependent in the range of 1 to 100 nM. The IC_{50} was approximately 10 nM. A TRAF-6 antisense oligonucleotide did not affect TRAF-2 mRNA expression.

The effect of oligonucleotide 16834 (SEQ ID NO. 54) on TRAF-2 protein levels was also examined. Cells were treated with oligonucleotide and allowed to recover for 48 to 72 hours before being harvested. Protein levels were determined by western blot analysis. Dose-dependent reduction of TRAF-2 protein expression was detectable 48 hours after treatment and maximal reduction of TRAF-2

protein levels was achieved 72 hours after treatment with 100 nM oligonucleotide.

TABLE 4

Activities of TRAF-2 Gapmer Oligonucleotides

ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
LIPOFECTIN only	---	---	100%	0%
16827	47	5'-UTR	43%	57%
16828	48	5'-UTR	23%	77%
16829	49	AUG	48%	52%
16830	50	AUG	18%	82%
16831	51	coding	49%	51%
16832	52	coding	42%	58%
16833	53	coding	60%	40%
16834	54	coding	3%	97%
16835	55	coding	43%	57%
16836	56	coding	91%	9%
16837	57	coding	60%	40%
16838	58	coding	66%	34%
16839	59	coding	47%	53%
16840	60	coding	45%	55%
16841	61	coding	8%	92%
16842	62	3'-UTR	36%	64%
16843	63	3'-UTR	46%	54%
16844	64	3'-UTR	82%	18%
16845	65	3'-UTR	59%	41%
16846	66	3'-UTR	13%	87%
16847	67	3'-UTR	74%	26%
16848	68	3'-UTR	57%	43%

ISIS 27693 (SEQ ID NO: 54) was also shown to decrease TRAF-2 mRNA levels in primary human fibroblast-like synoviocytes (obtained from surgical/biopsy specimens). LIPOFECTIN® was included at 3 μg/ml. A dose-response effect was obtained with an IC_{50} of approximately 25 nM and nearly 90% reduction of TRAF-2 mRNA at an oligonucleotide concentration of 100 nM.

Example 17:

Antisense inhibition of TRAF-3 expression- phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target human TRAF-3 RNA using published sequences (GenBank accession number HSU21092, SEQ ID NO: 3. Oligodeoxynucleotides are shown in Table 5. Target sites are indicated as nucleotide numbers on the TRAF-3 mRNA target (SEQ ID NO: 3).

TABLE 5

Nucleotide Sequences of Human TRAF-3 Phosphorothioate Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26778	AGAGCCGACGACCGCCGC	71	0078-0095	5'-UTR
26779	GGAAGAGCCGACGACCGC	72	0081-0098	5'-UTR
26780	CGCGCCAGGAGAGTCCAT	73	0236-0253	coding
26781	TTAGCGGCGGGTTAGTCT	74	0258-0275	coding
26782	AGCTTTAGCGGCGGGTTA	75	0262-0279	coding
26783	CTCGGTCTGCTTCGGGCT	76	0401-0418	coding
26784	TGCCCACACTCGGTCTGC	77	0409-0426	coding
26785	CGGTGCCCACACTCGGTC	78	0412-0429	coding
26786	GAAGCGGTGCCCACACTC	79	0416-0433	coding
26787	TTACACGCCTTCTCCACG	80	0712-0729	coding

	26788	GTATTTACACGCCTTCTC	81	0716-0733	coding
	26789	CCGGTATTTACACGCCTT	82	0719-0736	coding
	26790	GAGGGCAGGACACCACCA	83	0816-0833	coding
	26791	TGTGAGGGCAGGACACCA	84	0819-0836	coding
5	26792	CACTTGTGAGGGCAGGAC	85	0823-0840	coding
	26793	GCTGGTTTGTCCCCTGAA	86	0939-0956	coding
	26794	ATCTGCTGGTTTGTCCCC	87	0943-0960	coding
	26795	CGCGGTTCTGGAGGGACT	88	1281-1298	coding
	26796	CCCCGCACTCTTGTCCAC	89	1316-1333	coding
10	26797	TTGCCCCGCACTCTTGTC	90	1319-1336	coding
	26798	CCACTTGCCCCGCACTCT	91	1323-1340	coding
	26799	GAGCCACTTGCCCCGCAC	92	1326-1343	coding
	26800	TTCCGAGCCACTTGCCCC	93	1330-1347	coding
	26801	TCCGCCGCTTGTAGTCGC	94	1485-1502	coding
15	26802	TGCTTCCGCCGCTTGTAG	95	1489-1506	coding
	26803	TCCTGCTTCCGCCGCTTG	96	1492-1509	coding
	26804	GTCCCCGTTCAGGTAGAC	97	1589-1606	coding
	26805	TCCCGTCCCCGTTCAGGT	98	1593-1610	coding
	26806	CCATCCCGTCCCCGTTCA	99	1596-1613	coding
20	26807	TCCCCATCCCGTCCCCGT	100	1599-1616	coding
	26808	CCCTTCCCCATCCCGTCC	101	1603-1620	coding
	26809	TGCGTCCCCTTCCCCATC	102	1609-1626	coding
	26810	AAGTGCGTCCCCTTCCCC	103	1612-1629	coding
	26811	CGACAAGTGCGTCCCCTT	104	1616-1633	coding
25	26812	AAGGAAGCAGGGCATCAT	105	1662-1679	coding
	26813	CTCTCCAGTGGGCTTCTT	106	1781-1798	coding
	26814	TCATCTCTCCAGTGGGCT	107	1785-1802	coding
	26815	GCTAAATCCACCTCCCCA	108	1933-1950	3'-UTR
	26816	TCTGCCGCTTCCTCCGTC	109	2027-2044	3'-UTR
30	26817	CCGCCTTCTGCCGCTTCC	110	2033-2050	3'-UTR

[1] All cytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No.U21092, locus name "HSU21092" SEQ ID NO.3.

Example 18:

Antisense inhibition of TRAF-3 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a second series of oligonucleotides targeted to human TRAF-3 were synthesized. The oligonucleotide sequences are shown in Table 6. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. U21092), to which the oligonucleotide binds.

All compounds in Table 6 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines.

TABLE 6

Nucleotide Sequences of Human TRAF-3 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26818	AGAGCCGACGACCGCCGC	71	0078-0095	5'-UTR
26819	GGAAGAGCCGACGACCGC	72	0081-0098	5'-UTR
26820	CGCGCCAGGAGAGTCCAT	73	0236-0253	coding
26821	TTAGCGGCGGGTTAGTCT	74	0258-0275	coding
26822	AGCTTTAGCGGCGGGTTA	75	0262-0279	coding

	26823	CTCGGTCTGCTTCGGGCT	76	0401-0418	coding
	26824	TGCCCACACTCGGTCTGC	77	0409-0426	coding
	26825	CGGTGCCCACACTCGGTC	78	0412-0429	coding
	26826	GAAGCGGTGCCCACACTC	79	0416-0433	coding
5	26827	TTACACGCCTTCTCCACG	80	0712-0729	coding
	26828	GTATTTACACGCCTTCTC	81	0716-0733	coding
	26829	CCGGTATTTACACGCCTT	82	0719-0736	coding
	26830	GAGGGCAGGACACCACCA	83	0816-0833	coding
	26831	TGTGAGGGCAGGACACCA	84	0819-0836	coding
10	26832	CACTTGTGAGGGCAGGAC	85	0823-0840	coding
	26833	GCTGGTTTGTCCCCTGAA	86	0939-0956	coding
	26834	ATCTGCTGGTTTGTCCCC	87	0943-0960	coding
	26835	CGCGGTTCTGGAGGGACT	88	1281-1298	coding
	26836	CCCCGCACTCTTGTCCAC	89	1316-1333	coding
15	26837	TTGCCCCGCACTCTTGTC	90	1319-1336	coding
	26838	CCACTTGCCCCGCACTCT	91	1323-1340	coding
	26839	GAGCCACTTGCCCCGCAC	92	1326-1343	coding
	26840	TTCCGAGCCACTTGCCCC	93	1330-1347	coding
	26841	TCCGCCGCTTGTAGTCGC	94	1485-1502	coding
20	26842	TGCTTCCGCCGCTTGTAG	95	1489-1506	coding
	26843	TCCTGCTTCCGCCGCTTG	96	1492-1509	coding
	26844	GTCCCCGTTCAGGTAGAC	97	1589-1606	coding
	26845	TCCCGTCCCCGTTCAGGT	98	1593-1610	coding
	26846	CCATCCCGTCCCCGTTCA	99	1596-1613	coding
25	26847	TCCCCATCCCGTCCCCGT	100	1599-1616	coding
	26848	CCCTTCCCCATCCCGTCC	101	1603-1620	coding
	26849	TGCGTCCCCTTCCCCATC	102	1609-1626	coding
	26850	AAGTGCGTCCCCTTCCCC	103	1612-1629	coding
	26851	CGACAAGTGCGTCCCCTT	104	1616-1633	coding

26852	**AAGGAAGCAGGGCATCAT**	105	1662-1679	coding
26853	**CTCTCCAGTGGGCTTCTT**	106	1781-1798	coding
26854	**TCATCTCTCCAGTGGGCT**	107	1785-1802	coding
26855	**GCTAAATCCACCTCCCCA**	108	1933-1950	3'-UTR
26856	**TCTGCCGCTTCCTCCGTC**	109	2027-2044	3'-UTR
26857	**CCGCCTTCTGCCGCTTCC**	110	2033-2050	3'-UTR

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No. U21092, locus name "HSU21092" SEQ ID NO.3.

Example 19

Antisense inhibition of TRAF-4 expression- phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target different regions of the human TRAF-4 RNA, using published sequences (GenBank accession number X80200, incorporated herein as SEQ ID NO: 4). The oligonucleotides are shown in Table 7. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. X80200), to which the oligonucleotide binds. All compounds in Table 7 are oligodeoxynucleotides with phosphorothioate backbones (internucleoside linkages) throughout. The compounds are analyzed for effect on TRAF mRNA levels by quantitative real-time PCR as described in other examples herein.

TABLE 7

Nucleotide Sequences of Human TRAF-4 Phosphorothioate Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26860	GCATGGCGGGCGAGCGGC	111	0072-0089	AUG
26861	CCGTCGCTTGGGCTTCTC	112	0113-0130	coding
26862	GGGCACTTGAAGACTCCT	113	0232-0249	coding
26863	CTCAGGGCACTTGAAGAC	114	0236-0253	coding
26864	TGGTCCTCAGGGCACTTG	115	0241-0258	coding
26865	AAGCTGGTCCTCAGGGCA	116	0245-0262	coding
26866	GCGGCAGCCCTCCTCACT	117	0341-0358	coding
26867	CTCCAGCGGCAGCCCTCC	118	0346-0363	coding
26868	TAGGGCAGGGAATGACAT	119	0411-0428	coding
26869	CGATTAGGGCAGGGAATG	120	0415-0432	coding
26870	GGGCAGCGATTAGGGCAG	121	0421-0438	coding
26871	GCCTCCCCACTGAAGTCA	122	0523-0540	coding
26872	ATGCGGGCACCACACTTA	123	0592-0609	coding
26873	GGGCAGGCAACAGGCAGC	124	0733-0750	coding
26874	CCACAGTGCCCACACCAC	125	0759-0776	coding
26875	CGAGCCACAGTGCCCACA	126	0763-0780	coding
26876	TCCTCCCGAGCCACAGTG	127	0769-0786	coding
26877	CAGGTCCTCCCGAGCCAC	128	0773-0790	coding
26878	GGCAGAGCACCAGGGCGG	129	0819-0836	coding
26879	CTTTGAATGGGCAGAGCA	130	0828-0845	coding
26880	GGAGTCTTTGAATGGGCA	131	0833-0850	coding
26881	ATGCCGTGCCATTGCCAG	132	0875-0892	coding
26882	CTCACCAGGGCACACATC	133	0925-0942	coding
26883	CAGCTCCTGCCGTTGCCG	134	0944-0961	coding
26884	ATGAGCACGCCATCACTG	135	1000-1017	coding

	26885	TGTAGCCGCCGTCCATAG	136	1033-1050	coding
	26886	GCCTCCTGTAGCCGCCGT	137	1039-1056	coding
	26887	TAGAAGGCTGGGCTGAAG	138	1081-1098	coding
	26888	GTGTGTAGAAGGCTGGGC	139	1086-1103	coding
	26889	GTGTGCCCTCACCACTGC	140	1152-1169	coding
	26890	GACACGGCGGGCAAAGGG	141	1226-1243	coding
	26891	GAAGGTGACACGGCGGGC	142	1232-1249	coding
	26892	GCCCAGGGTCGCTCTGAT	143	1260-1277	coding
	26893	CTTCCAGTTTGGGTCGGG	144	1313-1330	coding
	26894	GATAACCAAAGCCCAGAG	145	1377-1394	coding
	26895	CATCGTCCTTTCCCCTCG	146	1513-1530	3'-UTR
	26896	GGCCAGGGCTGAAGCACC	147	1660-1677	3'-UTR
	26897	TTGTTTCCAGCCCTTCAT	148	1703-1720	3'-UTR
	26898	CATGTCTGCCCTACCCAA	149	1746-1763	3'-UTR
	26899	GCTCCCCTGCTGTGCCCT	150	1948-1965	3'-UTR

[1] All cytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No. X80200, locus name "HSMLN62" SEQ ID NO. 4.

Example 20:

Antisense inhibition of TRAF-4 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a second series of oligonucleotides targeted to human TRAF-4 were synthesized. The oligonucleotide sequences are shown in Table 8. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. X80200), to which the oligonucleotide binds.

All compounds in Table 8 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central

"gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines.

Data are obtained by real-time quantitative PCR as described in other examples herein.

TABLE 8

Nucleotide Sequences of Human TRAF-4 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26900	GCATGGCGGGCGAGCGGC	111	0072-0089	AUG
26901	CCGTCGCTTGGGCTTCTC	112	0113-0130	coding
26902	GGGCACTTGAAGACTCCT	113	0232-0249	coding
26903	CTCAGGGCACTTGAAGAC	114	0236-0253	coding
26904	TGGTCCTCAGGGCACTTG	115	0241-0258	coding
26905	AAGCTGGTCCTCAGGGCA	116	0245-0262	coding
26906	GCGGCAGCCCTCCTCACT	117	0341-0358	coding
26907	CTCCAGCGGCAGCCCTCC	118	0346-0363	coding
26908	TAGGGCAGGGAATGACAT	119	0411-0428	coding
26909	CGATTAGGGCAGGGAATG	120	0415-0432	coding
26910	GGGCAGCGATTAGGGCAG	121	0421-0438	coding
26911	GCCTCCCCACTGAAGTCA	122	0523-0540	coding
26912	ATGCGGGCACCACACTTA	123	0592-0609	coding
26913	GGGCAGGCAACAGGCAGC	124	0733-0750	coding
26914	CCACAGTGCCCACACCAC	125	0759-0776	coding
26915	CGAGCCACAGTGCCCACA	126	0763-0780	coding

26916	TCCTCCCGAGCCACAGTG	127	0769-0786	coding
26917	CAGGTCCTCCCGAGCCAC	128	0773-0790	coding
26918	GGCAGAGCACCAGGGCGG	129	0819-0836	coding
26919	CTTTGAATGGGCAGAGCA	130	0828-0845	coding
26920	GGAGTCTTTGAATGGGCA	131	0833-0850	coding
26921	ATGCCGTGCCATTGCCAG	132	0875-0892	coding
26922	CTCACCAGGGCACACATC	133	0925-0942	coding
26923	CAGCTCCTGCCGTTGCCG	134	0944-0961	coding
26924	ATGAGCACGCCATCACTG	135	1000-1017	coding
26925	TGTAGCCGCCGTCCATAG	136	1033-1050	coding
26926	GCCTCCTGTAGCCGCCGT	137	1039-1056	coding
26927	TAGAAGGCTGGGCTGAAG	138	1081-1098	coding
26928	GTGTGTAGAAGGCTGGGC	139	1086-1103	coding
26929	GTGTGCCCTCACCACTGC	140	1152-1169	coding
26930	GACACGGCGGGCAAAGGG	141	1226-1243	coding
26931	GAAGGTGACACGGCGGGC	142	1232-1249	coding
26932	GCCCAGGGTCGCTCTGAT	143	1260-1277	coding
26933	CTTCCAGTTTGGGTCGGG	144	1313-1330	coding
26934	GATAACCAAAGCCCAGAG	145	1377-1394	coding
26935	CATCGTCCTTTCCCCTCG	146	1513-1530	3'-UTR
26936	GGCCAGGGCTGAAGCACC	147	1660-1677	3'-UTR
26937	TTGTTTCCAGCCCTTCAT	148	1703-1720	3'-UTR
26938	CATGTCTGCCCTACCCAA	149	1746-1763	3'-UTR
26939	GCTCCCCTGCTGTGCCCT	150	1948-1965	3'-UTR

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No. X80200, locus name "HSMLN62" SEQ ID NO. 4.

Example 21

Antisense inhibition of TRAF-5 expression- phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target different regions of the human TRAF-5 RNA, using published sequences (GenBank accession number AB000509, incorporated herein as SEQ ID NO: 5). The oligonucleotides are shown in Table 9. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. AB000509), to which the oligonucleotide binds. All compounds in Table 9 are oligodeoxynucleotides with phosphorothioate backbones (internucleoside linkages) throughout.

TABLE 9

Nucleotide Sequences of Human TRAF-5 Phosphorothioate Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26940	TGAATAAGCCATTGTGGG	151	0049-0066	AUG
26941	CTTTATGCTCTTCTGAAT	152	0062-0079	coding
26942	GGATGAAACCACAGGGCA	153	0083-0100	coding
26943	TCAAAGTCCAAGGAAATG	154	0120-0137	coding
26944	TGAAGCACCGAGTGGCAG	155	0195-0212	coding
26945	GGGCAGATTGGCACTGTG	156	0282-0299	coding
26946	CTCCTGAGATTTGATGAC	157	0313-0330	coding
26947	CTTTCCGTAGGACTGGCT	158	0491-0508	coding
26948	GATTCTGTAGATTGATGA	159	0584-0601	coding
26949	TTCATCTACCTCAGTTTT	160	0667-0684	coding
26950	TCCGTTACAGCACAGCCA	161	0735-0752	coding
26951	GCATGTGCTCCCGTAAGG	162	0788-0805	coding
26952	CTTTTCAAGTTTCTTTAT	163	0907-0924	coding
26953	CTTCCATCAAAGGTCTCA	164	1079-1096	coding

26954	TCTAAAACGGCTAATCTT	165	1146-1163	coding
26955	TCATCTTGTAATCTGTCA	166	1283-1300	coding
26956	GGACTGGCTGAAGATGGA	167	1333-1350	coding
26957	CCCTCCCTGACCCATCCC	168	1403-1420	coding
26958	GAATGAGCCACAAAGCGG	169	1620-1637	coding
26959	CAAGAACAGAGTGTCATC	170	1672-1689	coding
26960	GTCTAAATCCAGGTCAAT	171	1799-1816	3'-UTR
26961	AAACTTACCATCTTTCAA	172	1964-1981	3'-UTR
26962	CTCTGTGTCCTCCATAAC	173	2053-2070	3'-UTR
26963	CTTAACTGGAACAGCCTA	174	2167-2184	3'-UTR
26964	GCAGGAAGAATGAAAATG	175	2352-2369	3'-UTR
26965	TATTTGGTTGAATCTTAT	176	2501-2518	3'-UTR
26966	AAATTCTATCCATCCTCA	177	2611-2628	3'-UTR
26967	AAATTGTAAAGGTTTTCT	178	2683-2700	3'-UTR
26968	ACAATGAAACTCTGTCTC	179	2779-2796	3'-UTR
26969	GCAAAACTCCGTCTCTAC	180	2940-2957	3'-UTR
26970	CAATAGTTGTCAGAGGCT	181	3055-3072	3'-UTR
26971	AAGGACTCATCTCAGTTT	182	3209-3226	3'-UTR
26972	TAACAACGCAGAAGGGCT	183	3280-3297	3'-UTR

	26973	AGTAGGGAAGTGGCATAA	184	3295-3312	3'-UTR
	26974	CATCACCAGGTAAGCAGC	185	3377-3394	3'-UTR
	26975	TCCTGTTGTGAACCTATT	186	3553-3570	3'-UTR
	26976	GGACTTGTGGGCTAAAGA	187	3656-3673	3'-UTR
5	26977	GCTCAGGAAGACAGAGTG	188	3724-3741	3'-UTR
	26978	TGAACTCCTAAGCAAACC	189	3873-3890	3'-UTR
	26979	GATGATGAAGGAACTCTG	190	3889-3906	3'-UTR

[1] All cytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

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[2] Coordinates from GenBank Accession No. AB000509, locus name "AB000509" SEQ ID NO. 5.

Example 22:

Antisense inhibition of TRAF-5 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a second series of oligonucleotides targeted to human TRAF-5 were synthesized. The oligonucleotide sequences are shown in Table 10. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. AB000509), to which the oligonucleotide binds.

All compounds in Table 10 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines.

TABLE 10

Nucleotide Sequences of Human TRAF-5 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
26980	TGAATAAGCCATTGTGGG	151	0049-0066	AUG
26981	CTTTATGCTCTTCTGAAT	152	0062-0079	coding
26982	GGATGAAACCACAGGGCA	153	0083-0100	coding
26983	TCAAAGTCCAAGGAAATG	154	0120-0137	coding
26984	TGAAGCACCGAGTGGCAG	155	0195-0212	coding
26985	GGGCAGATTGGCACTGTG	156	0282-0299	coding
26986	CTCCTGAGATTTGATGAC	157	0313-0330	coding
26987	CTTTCCGTAGGACTGGCT	158	0491-0508	coding
26988	GATTCTGTAGATTGATGA	159	0584-0601	coding
26989	TTCATCTACCTCAGTTTT	160	0667-0684	coding
26990	TCCGTTACAGCACAGCCA	161	0735-0752	coding
26991	GCATGTGCTCCCGTAAGG	162	0788-0805	coding
26992	CTTTTCAAGTTTCTTTAT	163	0907-0924	coding

26993	CTTCCATCAAAGGTCTCA	164	1079-1096	coding
26994	TCTAAAACGGCTAATCTT	165	1146-1163	coding
26995	TCATCTTGTAATCTGTCA	166	1283-1300	coding
26996	GGACTGGCTGAAGATGGA	167	1333-1350	coding
26997	CCCTCCCTGACCCATCCC	168	1403-1420	coding
26998	GAATGAGCCACAAAGCGG	169	1620-1637	coding
26999	CAAGAACAGAGTGTCATC	170	1672-1689	coding
27000	GTCTAAATCCAGGTCAAT	171	1799-1816	3'-UTR
27001	AAACTTACCATCTTTCAA	172	1964-1981	3'-UTR
27002	CTCTGTGTCCTCCATAAC	173	2053-2070	3'-UTR
27003	CTTAACTGGAACAGCCTA	174	2167-2184	3'-UTR
27004	GCAGGAAGAATGAAAATG	175	2352-2369	3'-UTR
27005	TATTTGGTTGAATCTTAT	176	2501-2518	3'-UTR
27006	AAATTCTATCCATCCTCA	177	2611-2628	3'-UTR
27007	AAATTGTAAAGGTTTTCT	178	2683-2700	3'-UTR
27008	ACAATGAAACTCTGTCTC	179	2779-2796	3'-UTR
27009	GCAAAACTCCGTCTCTAC	180	2940-2957	3'-UTR
27010	CAATAGTTGTCAGAGGCT	181	3055-3072	3'-UTR

27011	**AAG**GACTCATCTCA**GTTT**	182	3209-3226	3'-UTR
27012	**TAA**CAACGCAGAAG**GGCT**	183	3280-3297	3'-UTR
27013	**AGTA**GGGAAGTGGC**ATAA**	184	3295-3312	3'-UTR
27014	**CATC**ACCAGGT**/** AGCAGC	185	3377-3394	3'-UTR
27015	**TCCT**GTTGTGAACC**TATT**	186	3553-3570	3'-UTR
27016	**GGAC**TTGTGGGCTA**AAGA**	187	3656-3673	3'-UTR
27017	**GCTC**AGGAAGACAG**AGTG**	188	3724-3741	3'-UTR
27018	**TGAA**CTCCTAAGCA**AACC**	189	3873-3890	3'-UTR
27019	**GATG**ATGAAGGAAC**TCTG**	190	3889-3906	3'-UTR

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy-). All 2'-methoxyethoxy cytidines and 2'-deoxycytidines are 5-methyl-cytidines; all linkages are phosphorothioate linkages.

[2] Coordinates from GenBank Accession No. AB000509, locus name "AB000509" SEQ ID NO. 5.

Example 23:

Antisense inhibition of TRAF-6 expression- phosphorothioate 2'-MOE gapmer oligonucleotides

In accordance with the present invention, a series of oligonucleotides targeted to human TRAF-6 were synthesized. The oligonucleotide sequences are shown in Table 11. Target sites are indicated by nucleotide numbers, as given in the sequence source reference (GenBank accession no. U78798), to which the oligonucleotide binds.

All compounds in Table 11 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of eight 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by six-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) in the "deoxy gap" and phosphodiester (P=O) in the wings. Cytidine residues in the 2'-MOE wings are 5-methylcytidines.

TABLE 11:

Nucleotide Sequences of TRAF-6 Gapmer Oligonucleotides

ISIS NO.	NUCLEOTIDE SEQUENCE[1] (5' -> 3')	SEQ ID NO:	TARGET GENE NUCLEOTIDE COORDINATES[2]	GENE TARGET REGION
15779	AoGoGoCoCoAsAsGsCsCsCsCsAsGsCoToGoCoGoG	191	0001-0020	5'-UTR
15880	CoGoCoCoCoAoCsCsTsTsCsGsCsTsGsGoCoCoCoGoCoC	192	0024-0043	5'-UTR
15881	GoAoGoAoCoGsAsGsGsCsTsGsCsTsToGoGoAoCoG	193	0071-0090	5'-UTR
15882	GoGoAoCoCoAoCsAsGsAsCsAsCsTsGsCoGoCoGoCoC	194	0091-0110	5'-UTR
15883	CoCoCoAoAoGoGsCsGsCsTsGsGsTsAsGoCoAoGoGoAoC	195	0111-0130	5'-UTR
15884	ToToGoCoToCoCsGsTsTsCsTsAsGsTsGoCoGoCoCoGoG	196	0185-0204	5'-UTR
15885	CoAoToAoGoTsAsAsCsTsTsGsAsTsToAoToCoCoAoC	197	0205-0224	AUG
15886	AoGoCoCoAoGoAsCsTsCsCsAsTsAsGsTsAoAoCoCoToToG	198	0213-0232	AUG
15887	AoCoCoAoGoToToTsTsAsGsGsCsAsGsAsCsToCoCoAoToAoG	199	0220-0239	AUG
15888	AoCoCoAoGoCoCoGsCsTsAsCsCsAsGsGsAsGoCoToToGoGoC	200	0291-0310	coding
15889	AoToToToGoAoToTsTsTsTsAsTsGsGsAsTsGoCoCoAoGoGoC	201	0495-0514	coding
15890	GoToToGoAoCoCoCsTsGsGsCsCsAsTsCsCsCsToToAoToToG	202	0511-0530	coding
15891	GoToCoToCoCoAsGsGsTsTsCsCsCsAsTsCsToToGoToGoC	203	0641-0660	coding
15892	AoGoCoAoGoCoCoAsAsAsCsTsCsCsAsCsCsAsAoToGoToGoC	204	0678-0697	coding

15893	ToToToToGoGsAsAsGsGsGsAsCsGsCoToGoGoCoA	205	0714-0733	coding
15894	AoAoAoToGoCsCsAsTsTsGsAsTsGsCoAoGoCoAoC	206	0796-0815	coding
15895	AoToToCoAoCsAsGsAsTsGsAsCsAsToToToGoCoC	207	0851-0870	coding
15896	CoGoToGoCoCsAsAsGsTsGsAsTsTsCoCoToCoToG	208	0981-1000	coding
15897	GoGoToGoToTsCsTsCsTsTsGsTsAsGoGoToGoGoC	209	1000-1019	coding
15898	GoGoCoCoAoAsCsAsTsTsCsTsCsAsToGoToGoToG	210	1024-1043	coding
15899	CoGoCoToCoAsAsAsCsTsAsTsGsAsAoCoAoGoCoC	211	1046-1065	coding
15900	AoGoGoCoGoAsCsCsCsTsCsTsAsAsCoToGoGoToG	212	1119-1138	coding
15901	CoCoAoToToTsTsAsGsCsAsGsTsCsAoGoCoToCoC	213	1163-1182	coding
15902	CoGoAoAoToGsGsTsTsCsGsTsTsTsGoAoGoCoToC	214	1206-1225	coding
15903	CoCoAoToToGsCsAsCsTsGsCsTsGsToGoCoToToC	215	1254-1273	coding
15904	GoCoAoGoToCsGsGsTsAsAsCsTsGsAoAoGoGoToG	216	1401-1420	coding
15905	GoCoCoToToAsCsAsGsGsTsGsCsTsToCoAoGoAoC	217	1532-1551	coding
15906	AoGoCoAoAoGsCsAsGsCsTsCsTsGsGoToToToGoG	218	1576-1595	coding
15907	GoGoCoToAoCsCsCsAsTsGsTsCsAsAoAoGoCoGoG	219	1724-1743	coding
15908	ToToGoToToTsTsTsGsAsGsCsAsAsGoToGoAoGoG	220	1796-1815	3'-UTR
15909	GoGoCoAoCoTsGsTsTsTsTsCsTsCsCoAoGoGoToA	221	1817-1836	3'-UTR
15910	AoCoAoToAoTsTsTsCsCsCsGsTsGsGoCoToToGoT	222	1871-1890	3'-UTR

15911	GoGoAoAoCoGsTsGsTsGsGsAsTsTsCoCoCoAoGoG	223	1967-1986	3'-UTR
15912	ToGoCoToGoCsAsAsCsAsTsGsCsCsAoCoAoGoGoC	224	2017-2036	3'-UTR
15913	AoToAoCoAoCsCsAsGsAsGsCsAsAsAoAoGoCoCoC	225	2078-2097	3'-UTR
15914	AoAoAoAoGoAsCsTsGsAsAsCsTsTsToToAoAoGoG	226	scrambled control	
23247	AoCoToToAoAsTsTsAsCsCsAsTsGsAoCoToAoGoT	227	15910 mismatch	
23248	CoCoAoCoGoAsGsGsAsGsCsAsCsCsAoToCoAoAoG	228	16834 mismatch	
27691	AsCsAsTsAsTsTsTsCsCsCsGsTsGsGsCsTsTsGsT	222	1871-1890	3'-UTR
27692	AoCoAoToAsTsTsTsCsCsCsGsTsGsGsCoToToGoT	222	1871-1890	3'-UTR

[1] Emboldened residues are 2'-methoxyethoxy residues (others are 2'-deoxy). All 2'-methoxyethoxy cytidines are 5-methyl-cytidines, underlined "C" are 5-methyl-cytidine; "s" linkages are phosphorothioate linkages, "o" linkages are phosphodiester linkages.

[2] Coordinates from GenBank Accession No. U78798, locus name "HSU78798" SEQ ID NO. 6.

HMVEC cells were grown in 100 mm petri dishes until 70-80% confluent and then treated with oligonucleotide in the presence of cationic lipid. Briefly, cells were washed with PBS and OPTI-MEM®. OPTI-MEM® containing 10 μg/mL LIPOFECTIN® (Life Technologies, Rockville MD) was added to the cells, followed by addition of oligonucleotide. The cells were incubated for 3-4 hours at 37° C, washed once with EBM/1% FBS, and allowed to recover. For determination of mRNA levels by Northern blot, total RNA was prepared from cells by the guanidinium isothiocyanate procedure or by the Qiagen RNEASY™ method (Qiagen, Valencia, CA). Northern blot analysis was performed by standard methods (for example, Ausubel, et al. *Current Protocols in Molecular Biology*, Vol. 1, John Wiley and Sons, Inc., **1996**, pp.4.2.1-4.2.9). The probe was a PCR-labeled 1-kb fragment of TRAF-6 amplified by RT-PCR according to the method of Bednarczuk *et al.*, **1991**, Biotechniques *10*,478. RNA was quantified and normalized to G3PDH mRNA levels using a Molecular Dynamics (Sunnyvale, CA) PhosphorImager in accordance with manufacturer's instructions.

Results are shown in Table 12. Reduction of TRAF-6 mRNA levels with oligonucleotide 15910 (SEQ ID NO. 224) was determined to be dose-dependent in the range of 1 to 100 nM. The IC_{50} was approximately 2.5 nM. A TRAF-2 antisense oligonucleotide did not affect TRAF-6 mRNA expression.

TABLE 12

Activities of TRAF-6 Gapmer Oligonucleotides

ISIS No:	SEQ ID NO:	GENE TARGET REGION	% mRNA EXPRESSION	% mRNA INHIBITION
LIPOFECTIN® only	---	---	100%	0%
15779	191	5'-UTR	62%	38%

	15880	192	5'-UTR	73%	27%
	15881	193	5'-UTR	28%	72%
	15882	194	5'-UTR	96%	4%
	15883	195	5'-UTR	57%	43%
	15884	196	5'-UTR	73%	27%
	15885	197	AUG	61%	39%
	15886	198	AUG	37%	63%
	15887	199	AUG	23%	77%
	15888	200	coding	31%	69%
	15889	201	coding	42%	58%
	15890	202	coding	49%	51%
	15891	203	coding	50%	50%
	15892	204	coding	32%	68%
	15893	205	coding	18%	82%
	15894	206	coding	43%	57%
	15895	207	coding	41%	59%
	15896	208	coding	20%	80%
	15897	209	coding	60%	40%
	15898	210	coding	23%	77%
	15899	211	coding	66%	34%
	15900	212	coding	54%	46%
	15901	213	coding	60%	40%
	15902	214	coding	76%	24%
	15903	215	coding	58%	42%
	15904	216	coding	77%	23%
	15905	217	coding	108%	---
	15906	218	coding	90%	10%
	15907	219	coding	62%	38%
	15908	220	3'-UTR	82%	18%
	15909	221	3'-UTR	28%	72%

15910	222	3'-UTR	13%	87%
15911	223	3'-UTR	103%	---
15912	224	3'-UTR	20%	80%
15913	225	3'-UTR	97%	3%
15914	226	scrambled control	70%	30%

Example 24

Effect of inhibiting TRAF Gene Expression on the Induction of E-selectin

The effect of TRAF antisense oligonucleotides on the induction of E-selectin by TNFα or IL-1β was examined. HMVEC cells were treated with either ISIS 16834 or ISIS 15910 under dose-response conditions followed by stimulation of E-selectin expression by TNFα or IL-1β for 5 hours. The cell surface expression of E-selectin was determined by flow cytometry analysis. Dose-dependent inhibition of E-selectin cell surface induction by TNFα was observed in cells treated with the TRAF-2 antisense oligonucleotide ISIS 16834, as expected. Surprisingly, the TRAF-6 antisense compound, ISIS 15910, was able to inhibit TNFα mediated E-selectin surface expression as well, especially at higher dose. At low doses (20-50nM), ISIS 16834 was a more effective inhibitor of TNFα-mediated E-selectin induction than ISIS 15910. Maximal inhibition of E-selectin induction for both antisense compounds was approximately 70% at 100 nM. Control oligonucleotides exhibited little to no effect on E-selectin induction. When IL-1β was used as the stimulator, however, ISIS 15910 appeared to be a more specific and potent inhibitor of E-selectin induction than ISIS 16834, especially at relatively low doses.

Example 25

Effect of TRAF Antisense Oligonucleotide on Ikbα Phosphorylation and Degradation

Multiple transcription factors are activated by cytokines to facilitate the induction of E-selectin. The most important and best studied transcription factors involved in the regulation of E-selectin activation include NF-κB, c-Jun and ATF-2. To clarify the roles of TRAF proteins in the activation of NF-κB by cytokines, IκBα phosphorylation and degradation assays were performed with antisense oligonucleotide treated cells. Cells were treated with either ISIS 16834 or ISIS 15910 and allowed to recover for 48-72 hours. Tumor necrosis factor-α (TNF-α) or interleukin-1-β (IL-1β) was added for 5 to 30 minutes before cells were harvested. Western blot analysis with antibody specific for phospho-IκBα was performed to study the phosphorylation of IκBα. The blots were then stripped and reblotted with antibody against IκBα to study the degradation of IκBα. IκBα was heavily phosphorylated 5 minutes after addition of either cytokine. By 30 minutes, IκBα was reduced, probably as a result of IκBα degradation. In TNFα-stimulated cells, the majority of the IκBα had been degraded after minutes of stimulation. By 30 minutes, IκBα was almost completely gone. In contrast, the degradation of IκBα in IL-1β stimulated cells was slower with the majority of IκBα degraded by 30 minutes. Neither ISIS 16834 nor ISIS 15910 affected IκBα phosphorylation and degradation induced by TNFα. ISIS 15910 has little effect on IL-1β mediated IκBα phosphorylation and degradation either. Hyperphosphorylation of IκBα was observed in ISIS 16834 treated, IL-1β induced cells. In summary, the antisense oligonucleotides do not inhibit the phosphorylation and degradation of IκBα.

Example 26

Effect of TRAF Antisense Oligonucleotides on JNK Activities

MAP kinases play central roles in the activation of specific transcription factors crucial to the induction of cell adhesion molecules. To examine the effect of TRAF antisense oligonucleotides on JNK activities, *in vitro* kinase assays were performed on extracts derived from cells treated with TRAF antisense oligonucleotides. Cells were treated with TRAF-2 or TRAF-6 antisense compounds, (ISIS 16834 or ISIS 15910, respectively) allowed to recover for 48-72 hours, at which time TNF was added for 15 minutes prior to the cell lysis and the initiation of the kinase assays. Specific c-Jun conjugated agarose beads were used to precipitate JNK. ATP was added to the immunoprecipitated kinase complexes and the reaction mixes were analyzed on SDS-PAGE. Western blotting with antibodies specific for phosphorylated c-Jun was carried out to determine relative kinase activity. JNK was activated by TNFα after a 15 minute incubation, as indicated by the heavy phosphorylation of c-Jun. ISIS 16834 reduced JNK activity in TNFα-treated cells but not in IL-1β treated cells. Some hyperphosphorylation of c-Jun induced by IL-1β in ISIS 16834 treated cells was observed. ISIS 15910 reduced the c-Jun phosphorylation mediated by both IL-1β and TNFα. Some inhibitory effect of ISIS 15910 on JNK activity was also observed in TNFα-induced cells. This result is consistent with the inhibitory effects of TRAF antisense oligonucleotides on the surface expression of E-selectin.

Example 27

Inhibition of cell proliferation by antisense oligonucleotide targeted to TRAF-2

HeLa cells were treated with ISIS 16834 (200 nM) and counted 48 hours later. Cells were trypsinized, stained

with trypan blue and counted (floating cells included). Cell number in ISIS 16834-treated cultures was reduced by 61% compared to untreated control cultures.

Example 28

Induction of apoptosis by antisense oligonucleotide targeted to TRAF-2

HeLa cells were treated with ISIS 16834 (200 nM) and the number of dead cells was measured by trypan blue exclusion 48 hours later. In cultures untreated with oligonucleotide, only 5% of cells were dead. In cultures treated with ISIS 16834, 44% of cells were dead.

Example 29

Dose response of apoptosis in response to ISIS 16834 targeted to TRAF-2

HeLa cells were treated with ISIS 16834 at various doses and the number of sub-G1 apoptotic cells was counted using propidium iodide and FACS 48 hours after treatment with ISIS 16834 at 100, 200 and 300 nM. Culture supernatant and floating cells were transferred to FACS tubes. Cells were washed with PBS, trypsinized and washed in PBS, then fixed in ice-cold 70% ethanol for 12-15 hours in the freezer. Cells were centrifuged and resuspended in propidium iodide (PI) mix (50 μg/ml PI, 5 μg/ml RNAse cocktail, Cat. # 2286, Ambion, Austin TX) in the dark for 1 hour at room temperature before FACS analysis. Percent apoptotic cells after treatment at 100, 200 and 300 nM doses was approximately 29%, 46% and 58%, respectively, compared to 5% for untreated control cells.

Example 30

Time course of apoptosis in response to ISIS 16834 targeted to TRAF-2

The number of sub-G1 apoptotic HeLa cells was counted 1, 2 and 3 days after treatment with ISIS 16834 at 200 nM using methods described in the previous example. Percent

apoptotic cells after treatment at these time points was approximately 10%, 46% and 53%, respectively, compared to 5% after 3 days for untreated control cells.

Example 31

Antisense inhibition of TRAF-3 expression

The antisense oligonucleotides shown in Tables 5 and 6 were screened in T-24 cells for ability to inhibit human TRAF-3 expression. Results are shown in Tables 13 and 14, respectively.

Table 13

Inhibition of TRAF-3 mRNA levels by phosphorothioate oligodeoxynucleotides

	ISIS#	TARGET REGION	SITE[1]	SEQUENCE 5'--> 3'	%Inhib	SEQ ID NO.
	26778	5'UTR	78	AGAGCCGACGACCGCCGC	0	71
	26779	5'UTR	81	GGAAGAGCCGACGACCGC	0	72
	26780	CDS	236	CGCGCCAGGAGAGTCCAT	0	73
	26781	CDS	258	TTAGCGGCGGGTTAGTCT	22	74
	26782	CDS	262	AGCTTTAGCGGCGGGTTA	9	75
	26783	CDS	401	CTCGGTCTGCTTCGGGCT	30	76
	26784	CDS	409	TGCCCACACTCGGTCTGC	67	77
	26785	CDS	412	CGGTGCCCACACTCGGTC	70	78
	26786	CDS	416	GAAGCGGTGCCCACACTC	66	79
	26787	CDS	712	TTACACGCCTTCTCCACG	77	80
	26788	CDS	716	GTATTTACACGCCTTCTC	25	81
	26789	CDS	719	CCGGTATTTACACGCCTT	35	82
	26790	CDS	816	GAGGGCAGGACACCACCA	81	83
	26791	CDS	819	TGTGAGGGCAGGACACCA	70	84
	26792	CDS	823	CACTTGTGAGGGCAGGAC	69	85
	26793	CDS	939	GCTGGTTTGTCCCCTGAA	42	86
	26794	CDS	943	ATCTGCTGGTTTGTCCCC	73	87
	26795	CDS	1281	CGCGGTTCTGGAGGGACT	39	88
	26796	CDS	1316	CCCCGCACTCTTGTCCAC	36	89
	26797	CDS	1319	TTGCCCCGCACTCTTGTC	19	90
	26798	CDS	1323	CCACTTGCCCCGCACTCT	70	91
	26799	CDS	1326	GAGCCACTTGCCCCGCAC	53	92
	26800	CDS	1330	TTCCGAGCCACTTGCCCC	22	93
	26801	CDS	1485	TCCGCCGCTTGTAGTCGC	70	94
	26802	CDS	1489	TGCTTCCGCCGCTTGTAG	42	95
	26803	CDS	1492	TCCTGCTTCCGCCGCTTG	73	96
	26804	CDS	1589	GTCCCCGTTCAGGTAGAC	43	97
	26805	CDS	1593	TCCCGTCCCCGTTCAGGT	78	98

26806	CDS	1596	CCATCCCGTCCCCGTTCA	81	99
26807	CDS	1599	TCCCCATCCCGTCCCCGT	86	100
26808	CDS	1603	CCCTTCCCCATCCCGTCC	40	101
26809	CDS	1609	TGCGTCCCCTTCCCCATC	45	102
26810	CDS	1612	AAGTGCGTCCCCTTCCCC	32	103
26811	CDS	1616	CGACAAGTGCGTCCCCTT	71	104
26812	CDS	1662	AAGGAAGCAGGGCATCAT	44	105
26813	CDS	1781	CTCTCCAGTGGGCTTCTT	70	106
26814	CDS	1785	TCATCTCTCCAGTGGGCT	48	107
26815	3'UTR	1933	GCTAAATCCACCTCCCCA	0	108
26816	3'UTR	2027	TCTGCCGCTTCCTCCGTC	41	109
26817	3'UTR	2033	CCGCCTTCTGCCGCTTCC	70	110

[1]Position of first nucleotide of the target site on GenBank accession number HSU21092, incorporated herein as SEQ ID NO: 3.

Table 14

Inhibition of TRAF-3 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS#	TARGET REGION	SITE[1]	SEQUENCE 5'--> 3'	%Inhib	SEQ ID NO.
26818	5'UTR	78	AGAGCCGACGACCGCCGC	2	71
26819	5'UTR	81	GGAAGAGCCGACGACCGC	0	72
26820	CDS	236	CGCGCCAGGAGAGTCCAT	87	73
26821	CDS	258	TTAGCGGCGGGTTAGTCT	32	74
26822	CDS	262	AGCTTTAGCGGCGGGTTA	33	75
26823	CDS	401	CTCGGTCTGCTTCGGGCT	58	76
26824	CDS	409	TGCCCACACTCGGTCTGC	71	77
26825	CDS	412	CGGTGCCCACACTCGGTC	69	78
26826	CDS	416	GAAGCGGTGCCCACACTC	54	79
26827	CDS	712	TTACACGCCTTCTCCACG	65	80
26828	CDS	716	GTATTTACACGCCTTCTC	40	81
26829	CDS	719	CCGGTATTTACACGCCTT	77	82
26830	CDS	816	GAGGGCAGGACACCACCA	76	83
26831	CDS	819	TGTGAGGGCAGGACACCA	78	84
26832	CDS	823	CACTTGTGAGGGCAGGAC	88	85
26833	CDS	939	GCTGGTTTGTCCCCTGAA	79	86
26834	CDS	943	ATCTGCTGGTTTGTCCCC	74	87

	26835	CDS	1281	CGCGGTTCTGGAGGGACT	57	88
	26836	CDS	1316	CCCCGCACTCTTGTCCAC	0	89
	26837	CDS	1319	TTGCCCCGCACTCTTGTC	34	90
	26838	CDS	1323	CCACTTGCCCCGCACTCT	33	91
5	26839	CDS	1326	GAGCCACTTGCCCCGCAC	39	92
	26840	CDS	1330	TTCCGAGCCACTTGCCCC	0	93
	26841	CDS	1485	TCCGCCGCTTGTAGTCGC	71	94
	26842	CDS	1489	TGCTTCCGCCGCTTGTAG	39	95
	26843	CDS	1492	TCCTGCTTCCGCCGCTTG	47	96
10	26844	CDS	1589	GTCCCCGTTCAGGTAGAC	7	97
	26845	CDS	1593	TCCCGTCCCCGTTCAGGT	56	98
	26846	CDS	1596	CCATCCCGTCCCCGTTCA	54	99
	26847	CDS	1599	TCCCCATCCCGTCCCCGT	41	100
	26848	CDS	1603	CCCTTCCCCATCCCGTCC	79	101
15	26849	CDS	1609	TGCGTCCCCTTCCCCATC	63	102
	26850	CDS	1612	AAGTGCGTCCCCTTCCCC	77	103
	26851	CDS	1616	CGACAAGTGCGTCCCCTT	80	104
	26852	CDS	1662	AAGGAAGCAGGGCATCAT	4	105
	26853	CDS	1781	CTCTCCAGTGGGCTTCTT	64	106
20	26854	CDS	1785	TCATCTCTCCAGTGGGCT	55	107
	26855	3'UTR	1933	GCTAAATCCACCTCCCCA	48	108
	26856	3'UTR	2027	TCTGCCGCTTCCTCCGTC	39	109

26857	3'UTR	2033	CCGCCTTCTGCCGCTTCC	65	110

[1]Position of first nucleotide of the target site on GenBank accession number HSU21092, incorporated herein as SEQ ID NO: 3.

Example 32

Antisense inhibition of TRAF-4 expression

The antisense oligonucleotides shown in Tables 7 and 8 were screened in T-24 cells for ability to inhibit human TRAF-4 expression. Results are shown in Tables 15 and 16, respectively.

Table 15

Inhibition of TRAF-4 mRNA levels by phosphorothioate oligodeoxynucleotides

	ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
5	26860	Start	72	GCATGGCGGGCGAGCGGC	0	111
	26861	CDS	113	CCGTCGCTTGGGCTTCTC	16	112
	26862	CDS	232	GGGCACTTGAAGACTCCT	0	113
	26863	CDS	236	CTCAGGGCACTTGAAGAC	0	114
	26864	CDS	241	TGGTCCTCAGGGCACTTG	47	115
10	26865	CDS	245	AAGCTGGTCCTCAGGGCA	0	116
	26866	CDS	341	GCGGCAGCCCTCCTCACT	6	117
	26867	CDS	346	CTCCAGCGGCAGCCCTCC	61	118
	26868	CDS	411	TAGGGCAGGGAATGACAT	0	119
	26869	CDS	415	CGATTAGGGCAGGGAATG	20	120
15	26870	CDS	421	GGGCAGCGATTAGGGCAG	54	121
	26871	CDS	523	GCCTCCCCACTGAAGTCA	38	122
	26872	CDS	592	ATGCGGGCACCACACTTA	56	123
	26873	CDS	733	GGGCAGGCAACAGGCAGC	53	124
	26874	CDS	759	CCACAGTGCCCACACCAC	34	125
20	26875	CDS	763	CGAGCCACAGTGCCCACA	43	126
	26876	CDS	769	TCCTCCCGAGCCACAGTG	66	127
	26877	CDS	773	CAGGTCCTCCCGAGCCAC	35	128
	26878	CDS	819	GGCAGAGCACCAGGGCGG	54	129

	26879	CDS	828	CTTTGAATGGGCAGAGCA	48	130
	26880	CDS	833	GGAGTCTTTGAATGGGCA	0	131
	26881	CDS	875	ATGCCGTGCCATTGCCAG	44	132
	26882	CDS	925	CTCACCAGGGCACACATC	69	133
5	26883	CDS	944	CAGCTCCTGCCGTTGCCG	71	134
	26884	CDS	1000	ATGAGCACGCCATCACTG	0	135
	26885	CDS	1033	TGTAGCCGCCGTCCATAG	74	136
	26886	CDS	1039	GCCTCCTGTAGCCGCCGT	53	137
	26887	CDS	1081	TAGAAGGCTGGGCTGAAG	48	138
10	26888	CDS	1086	GTGTGTAGAAGGCTGGGC	23	139
	26889	CDS	1152	GTGTGCCCTCACCACTGC	32	140
	26890	CDS	1226	GACACGGCGGGCAAAGGG	52	141
	26891	CDS	1232	GAAGGTGACACGGCGGGC	73	142
	26892	CDS	1260	GCCCAGGGTCGCTCTGAT	80	143
15	26893	CDS	1313	CTTCCAGTTTGGGTCGGG	0	144
	26894	CDS	1377	GATAACCAAAGCCCAGAG	45	145
	26895	3'UTR	1513	CATCGTCCTTTCCCCTCG	51	146
	26896	3'UTR	1660	GGCCAGGGCTGAAGCACC	53	147
	26897	3'UTR	1703	TTGTTTCCAGCCCTTCAT	67	148
20	26898	3'UTR	1746	CATGTCTGCCCTACCCAA	0	149
	26899	3'UTR	1948	GCTCCCCTGCTGTGCCCT	49	150

[1]Position of first nucleotide of the target site on GenBank Accession No. X80200, locus name "HSMLN62" SEQ ID NO. 4.

Table 16

Inhibition of TRAF-4 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26901	CDS	113	CCGTCGCTTGGGCTTCTC	25	112
26902	CDS	232	GGGCACTTGAAGACTCCT	57	113
26903	CDS	236	CTCAGGGCACTTGAAGAC	49	114
26904	CDS	241	TGGTCCTCAGGGCACTTG	0	115
26905	CDS	245	AAGCTGGTCCTCAGGGCA	0	116
26906	CDS	341	GCGGCAGCCCTCCTCACT	0	117
26907	CDS	346	CTCCAGCGGCAGCCCTCC	68	118
26908	CDS	411	TAGGGCAGGGAATGACAT	0	119
26909	CDS	415	CGATTAGGGCAGGGAATG	0	120
26910	CDS	421	GGGCAGCGATTAGGGCAG	39	121
26911	CDS	523	GCCTCCCCACTGAAGTCA	30	122
26912	CDS	592	ATGCGGGCACCACACTTA	44	123
26913	CDS	733	GGGCAGGCAACAGGCAGC	66	124
26914	CDS	759	CCACAGTGCCCACACCAC	43	125
26915	CDS	763	CGAGCCACAGTGCCCACA	6	126
26916	CDS	769	TCCTCCCGAGCCACAGTG	46	127
26917	CDS	773	CAGGTCCTCCCGAGCCAC	75	128

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26918	CDS	819	GGCAGAGCACCAGGGCGG	74	129
26919	CDS	828	CTTTGAATGGGCAGAGCA	34	130
26920	CDS	833	GGAGTCTTTGAATGGGCA	11	131
26921	CDS	875	ATGCCGTGCCATTGCCAG	32	132
26922	CDS	925	CTCACCAGGGCACACATC	30	133
26923	CDS	944	CAGCTCCTGCCGTTGCCG	79	134
26924	CDS	1000	ATGAGCACGCCATCACTG	39	135
26925	CDS	1033	TGTAGCCGCCGTCCATAG	24	136
26926	CDS	1039	GCCTCCTGTAGCCGCCGT	61	137
26927	CDS	1081	TAGAAGGCTGGGCTGAAG	51	138
26928	CDS	1086	GTGTGTAGAAGGCTGGGC	75	139
26929	CDS	1152	GTGTGCCCTCACCACTGC	23	140
26930	CDS	1226	GACACGGCGGGCAAAGGG	27	141
26931	CDS	1232	GAAGGTGACACGGCGGGC	65	142
26932	CDS	1260	GCCCAGGGTCGCTCTGAT	76	143
26933	CDS	1313	CTTCCAGTTTGGGTCGGG	0	144
26934	CDS	1377	GATAACCAAAGCCCAGAG	0	145
26935	3'UTR	1513	CATCGTCCTTTCCCCTCG	11	146
26936	3'UTR	1660	GGCCAGGGCTGAAGCACC	79	147
26937	3'UTR	1703	TTGTTTCCAGCCCTTCAT	7	148

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26938	3'UTR	1746	CATGTCTGCCCTACCCAA	26	149
26939	3'UTR	1948	GCTCCCCTGCTGTGCCCT	14	150

[1]Position of first nucleotide of the target site on GenBank Accession No. X80200, locus name "HSMLN62" SEQ ID NO. 4.

Example 32

Antisense inhibition of TRAF-5 expression

The antisense oligonucleotides shown in Tables 9 and 10 were screened in T-24 cells for ability to inhibit human TRAF-5 expression. Results are shown in Tables 17 and 18, respectively.

Table 17

Inhibition of TRAF-5 mRNA levels by phosphorothioate oligodeoxynucleotides

	ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
5	26940	Start	49	TGAATAAGCCATTGTGGG	33	151
	26941	CDS	62	CTTTATGCTCTTCTGAAT	0	152
	26942	CDS	83	GGATGAAACCACAGGGCA	14	153
	26943	CDS	120	TCAAAGTCCAAGGAAATG	37	154
	26944	CDS	195	TGAAGCACCGAGTGGCAG	37	155
10	26945	CDS	282	GGGCAGATTGGCACTGTG	79	156
	26946	CDS	313	CTCCTGAGATTTGATGAC	0	157
	26947	CDS	491	CTTTCCGTAGGACTGGCT	60	158
	26948	CDS	584	GATTCTGTAGATTGATGA	2	159
	26949	CDS	667	TTCATCTACCTCAGTTTT	55	160
15	26950	CDS	735	TCCGTTACAGCACAGCCA	59	161
	26951	CDS	788	GCATGTGCTCCCGTAAGG	77	162
	26952	CDS	907	CTTTTCAAGTTTCTTTAT	4	163
	26953	CDS	1079	CTTCCATCAAAGGTCTCA	35	164
	26954	CDS	1146	TCTAAAACGGCTAATCTT	0	165
20	26955	CDS	1283	TCATCTTGTAATCTGTCA	9	166
	26956	CDS	1333	GGACTGGCTGAAGATGGA	7	167
	26957	CDS	1403	CCCTCCCTGACCCATCCC	71	168

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26958	CDS	1620	GAATGAGCCACAAAGCGG	68	169
26959	CDS	1672	CAAGAACAGAGTGTCATC	13	170
26960	3'UTR	1799	GTCTAAATCCAGGTCAAT	26	171
26961	3'UTR	1964	AAACTTACCATCTTTCAA	48	172
26962	3'UTR	2053	CTCTGTGTCCTCCATAAC	54	173
26963	3'UTR	2167	CTTAACTGGAACAGCCTA	35	174
26964	3'UTR	2352	GCAGGAAGAATGAAAATG	0	175
26965	3'UTR	2501	TATTTGGTTGAATCTTAT	8	176
26966	3'UTR	2611	AAATTCTATCCATCCTCA	32	177
26967	3'UTR	2683	AAATTGTAAAGGTTTTCT	22	178
26968	3'UTR	2779	ACAATGAAACTCTGTCTC	14	179
26969	3'UTR	2940	GCAAAACTCCGTCTCTAC	51	180
26970	3'UTR	3055	CAATAGTTGTCAGAGGCT	39	181
26971	3'UTR	3209	AAGGACTCATCTCAGTTT	0	182
26972	3'UTR	3280	TAACAACGCAGAAGGGCT	74	183
26973	3'UTR	3295	AGTAGGGAAGTGGCATAA	29	184
26974	3'UTR	3377	CATCACCAGGTAAGCAGC	60	185
26975	3'UTR	3553	TCCTGTTGTGAACCTATT	40	186
26976	3'UTR	3656	GGACTTGTGGGCTAAAGA	60	187
26977	3'UTR	3724	GCTCAGGAAGACAGAGTG	6	188

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26978	3'UTR	3873	TGAACTCCTAAGCAAACC	23	189
26979	3'UTR	3889	GATGATGAAGGAACTCTG	20	190

[1]Position of first nucleotide of the target site on GenBank GenBank Accession No. AB000509, locus name "AB000509" SEQ ID NO. 5

Table 18

Inhibition of TRAF-5 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26980	Start	49	TGAAT[illegible]GCCATTGTGGG	27	151
26981	CDS	62	CTTTATGCTCTTCTGAAT	43	152
26982	CDS	83	GGATGAAACCACAGGGCA	71	153
26983	CDS	120	TCAAAGTCCAAGGAAATG	48	154
26984	CDS	195	TGAAGCACCGAGTGGCAG	66	155
26985	CDS	282	GGGCAGATTGGCACTGTG	37	156
26986	CDS	313	CTCCTGAGATTTGATGAC	64	157
26987	CDS	491	CTTTCCGTAGGACTGGCT	71	158
26988	CDS	584	GATTCTGTAGATTGATGA	18	159
26989	CDS	667	TTCATCTACCTCAGTTTT	50	160
26990	CDS	735	TCCGTTACAGCACAGCCA	66	161
26991	CDS	788	GCATGTGCTCCCGTAAGG	83	162
26992	CDS	907	CTTTTCAAGTTTCTTTAT	39	163
26993	CDS	1079	CTTCCATCAAAGGTCTCA	82	164
26994	CDS	1146	TCTAAAACGGCTAATCTT	38	165
26995	CDS	1283	TCATCTTGTAATCTGTCA	61	166
26996	CDS	1333	GGACTGGCTGAAGATGGA	35	167

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
26997	CDS	1403	CCCTCCCTGACCCATCCC	40	168
26998	CDS	1620	GAATGAGCCACAAAGCGG	76	169
26999	CDS	1672	CAAGAACAGAGTGTCATC	25	170
27000	3'UTR	1799	GTCTAAATCCAGGTCAAT	30	171
27001	3'UTR	1964	AAACTTACCATCTTTCAA	66	172
27002	3'UTR	2053	CTCTGTGTCCTCCATAAC	68	173
27003	3'UTR	2167	CTTAACTGGAACAGCCTA	68	174
27004	3'UTR	2352	GCAGGAAGAATGAAAATG	20	175
27005	3'UTR	2501	TATTTGGTTGAATCTTAT	38	176
27006	3'UTR	2611	AAATTCTATCCATCCTCA	0	177
27007	3'UTR	2683	AAATTGTAAAGGTTTTCT	8	178
27008	3'UTR	2779	ACAATGAAACTCTGTCTC	66	179
27009	3'UTR	2940	GCAAAACTCCGTCTCTAC	51	180
27010	3'UTR	3055	CAATAGTTGTCAGAGGCT	32	181
27011	3'UTR	3209	AAGGACTCATCTCAGTTT	20	182
27012	3'UTR	3280	TAACAACGCAGAAGGGCT	64	183
27013	3'UTR	3295	AGTAGGGAAGTGGCATAA	58	184
27014	3'UTR	3377	CATCACCAGGTAAGCAGC	59	185
27015	3'UTR	3553	TCCTGTTGTGAACCTATT	79	186
27016	3'UTR	3656	GGACTTGTGGGCTAAAGA	67	187

ISIS#	TARGET	SITE[1]	SEQUENCE	%Inhib	SEQ ID
27017	3'UTR	3724	GCTCAGGAAGACAGAGTG	62	188
27018	3'UTR	3873	TGAACTCCTAAGCAAACC	16	189
27019	3'UTR	3889	GATGATGAAGGAACTCTG	52	190

[1]Position of first nucleotide of the target site on GenBank Accession No. AB000509, locus name "AB000509" SEQ ID NO. 5.

Example 33

Additional oligonucleotides targeted to TRAF-1

Additional antisense oligonucleotides targeted to TRAF-1 were designed and synthesized. All compounds are 2'-MOE gapmers with 2'-MOE nucleotides shown in bold. Backbones are phosphorothioates throughout. All C are 5-methyl C. Compounds were tested as described in Example 13 above. Compounds and results are shown in Table 19.

Table 19

Antisense inhibition of TRAF-1 expression

ISIS #	SEQUENCE	GENE TARGET REGION	START POS[1]	% INHIB	SEQ ID NO:
101869	**GGACC**AGCCTTGTGG**AGTCC**	5' UTR	5	--	229
101870	**TCAGG**GTTCCAGGCT**GGCCA**	5' UTR	55	--	230
101871	**TCTCA**GGGTTCCAGG**CTGGC**	Start	57	51	231
101872	**CATCT**CAGGGTTCCA**GGCTG**	Start	59	50	232
101873	**GCCAT**CTCAGGGTTC**CAGGC**	Start	61	5	233
101874	**AGGCC**ATCTCAGGGT**TCCAG**	Start	63	--	234
101875	**GGAGG**CCATCTCAGG**GTTCC**	Start	65	--	235
101876	**CTGGA**GGCCATCTCA**GGGTT**	Start	67	7	236
101877	**AGCTG**GAGGCCATCT**CAGGG**	Start	69	--	237

	ISIS #	SEQUENCE	GENE TARGET REGION	START POS[1]	% INHIB	SEQ ID NO:
	101878	**TGAGC**TGGAGGCCAT**CTCAG**	Start	71	--	238
	101879	**CCTGA**GCTGGAGGCC**ATCTC**	Start	73	28	239
	101880	**TGCCT**GAGCTGGAGG**CCATC**	Start	75	--	240
	101881	**GCTGC**CTGAGCTGGA**GGCCA**	Start	77	--	241
5	101882	**GGGCG**AGGACTGCTG**CCTGA**	Coding	88	60	242
	101883	**TCTCA**GAGAGACAGC**CTGCA**	Coding	189	43	243
	101884	**TCCTG**GGCTTATAGA**CTGGA**	Coding	260	78	244
	101885	**GGGCT**TCCCTTGAAG**GAGCA**	Coding	358	--	245
	101886	**CAACA**GCAGGTTTAG**GTGGG**	Coding	416	--	246
10	101887	**CAGGG**CCATGGGCCC**AGACT**	Coding	479	--	247
	101888	**CCCGG**TAGCAATCGA**CCTCC**	Coding	555	31	248
	101889	**CTCAG**CCAGAAGCTT**CTCCT**	Coding	623	53	249
	101890	**GGCCA**GGGCCAGGTG**GGAGG**	Coding	704	--	250
	101891	**TGCTG**AAGCTCCACC**ACCCT**	Coding	778	11	251
15	101892	**GTGAT**CTTCCACAGG**AAAGT**	Coding	877	8	252
	101893	**TTGGC**AGTGTAGAAG**GCTGG**	Coding	955	27	253
	101894	**AAGAG**CGACAGATGG**GTTCT**	Coding	1030	61	254
	101895	**GCAGC**ATGAAGGTGA**CCTTG**	Coding	1104	--	255
	101896	**TCTGG**AAGGACGCTG**AGCTT**	Coding	1173	--	256
20	101897	**CTCCA**CAATGCACTT**GAGGA**	Coding	1295	35	257
	101898	**TGCCC**TGAGGTCTTG**GGTGC**	3' UTR	1399	31	258
	101899	**ATCCT**AACCAGATGG**CCAGC**	3' UTR	1473	70	259
	101900	**ACTGG**CCTCCCAGTG**TCGCA**	3' UTR	1571	9	260
	101901	**GCTTG**GGTCCTACGG**TTCCA**	3' UTR	1656	22	261
25	101902	**TCCTG**TTTCTGACCC**TGGAG**	3' UTR	1749	81	262
	101903	**CTGGG**TTTGCTTGTT**CACCT**	3' UTR	1826	85	263
	101904	**CCAGG**AGGCTAGAAT**GAGAG**	3' UTR	1917	--	264
	101905	**TGAGG**AGCTGGGAGG**ACAGG**	3' UTR	1986	--	265
	101906	**TTGGG**AAGCTGAGCT**GCCAG**	3' UTR	2066	--	266

ISIS #	SEQUENCE	GENE TARGET REGION	START POS[1]	% INHIB	SEQ ID NO:
101907	**ACAGT**GACTGGGTTT**CACCT**	3' UTR	2153	35	267
101908	**TTACG**GGATTCTGGA**AAGCA**	3' UTR	2256	78	268

[1]Position of first nucleotide of the target site on GenBank Accession No.U19261, locus name "HSU19261," SEQ ID NO: 1.

What is claimed is:

1. An antisense compound 8 to 30 nucleotides in length targeted to a nucleic acid molecule encoding a human tumor necrosis factor receptor-associated factor, wherein said antisense compound inhibits the expression of human tumor necrosis factor receptor-associated factor.

2. The antisense compound of claim 1 which is an antisense oligonucleotide.

3. The antisense oligonucleotide of claim 2 which comprises at least one modified internucleoside linkage.

4. The antisense oligonucleotide of claim 3 wherein the modified internucleoside linkage is a phosphorothioate linkage.

5. The antisense oligonucleotide of claim 2 which comprises at least one modified sugar moiety.

6. The antisense oligonucleotide of claim 5 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety.

7. The antisense oligonucleotide of claim 2 which comprises at least one modified nucleobase.

8. The antisense oligonucleotide of claim 7 wherein the modified nucleobase is a 5-methylcytosine.

9. The antisense compound of claim 2 which is a chimeric oligonucleotide.

10. The antisense compound of claim 1 wherein the human TRAF is TRAF-2 or TRAF-6.

11. A composition comprising the antisense compound of claim 1 and a pharmaceutically acceptable carrier or diluent.

12. The composition of claim 11 comprising a colloidal dispersion system.

13. The composition of claim 11 wherein the antisense compound is an antisense oligonucleotide.

14. A method of inhibiting the expression of tumor necrosis factor receptor-associated factor in human cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that expression of tumor necrosis factor receptor-associated factor is inhibited.

15. A method of treating a human having a disease or condition associated with tumor necrosis factor receptor-associated factor comprising administering to said animal a therapeutically or prophylactically effective amount of the antisense compound of claim 1 so that expression of tumor necrosis factor receptor-associated factor is inhibited.

16. The method of claim 15 wherein the disease or condition is a hyperproliferative or inflammatory disease or condition.

17. A method of reducing jun kinase activation in cells or tissues by tumor necrosis factor-α comprising contacting said cells or tissues with an antisense compound targeted to TRAF-2.

18. A method of reducing jun kinase activation in cells

or tissues comprising contacting said cells or tissues with an antisense compound targeted to TRAF-6.

19. A method of reducing E-selectin expression in cells or tissues comprising contacting said cells or tissues with an antisense compound targeted to TRAF-2 or TRAF-6.

SEQUENCE LISTING

<110> Baker, Brenda F.
Cowsert, Lex M.
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Xu, Xiaoxing S.
Isis Pharmaceuticals, Inc.

<120> ANTISENSE MODULATION OF EXPRESSION OF TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTOR (TRAFs)

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Met Ala Ser Ser Ser Gly Ser Ser Pro Arg Pro Ala
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Pro Asp Glu Asn Glu Phe Pro Phe Gly Cys Pro Pro Thr Val Cys Gln
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Asp Pro Lys Glu Pro Arg Ala Leu Cys Cys Ala Gly Cys Leu Ser Glu
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aac ccg agg aat ggc gag gat cag atc tgc ccc aaa tgc aga ggg gaa 255
Asn Pro Arg Asn Gly Glu Asp Gln Ile Cys Pro Lys Cys Arg Gly Glu
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Asp Leu Gln Ser Ile Ser Pro Gly Ser Arg Leu Arg Thr Gln Glu Lys
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Ala His Pro Glu Val Ala Glu Ala Gly Ile Gly Cys Pro Phe Ala Gly
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gtc ggc tgc tcc ttc aag gga agc cca cag tct gtg caa gag cat gag 399
Val Gly Cys Ser Phe Lys Gly Ser Pro Gln Ser Val Gln Glu His Glu
95 100 105

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gtc acc tcc cag acc tcc cac cta aac ctg ctg ttg ggg ttc atg aaa   447
Val Thr Ser Gln Thr Ser His Leu Asn Leu Leu Leu Gly Phe Met Lys
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cag tgg aag gcc cgg ctg ggc tgt ggc ctg gag tct ggg ccc atg gcc   495
Gln Trp Lys Ala Arg Leu Gly Cys Gly Leu Glu Ser Gly Pro Met Ala
125                 130                 135                 140

ctg gag cag aac ctg tca gac ctg cag ctg cag gca gcc gtg gaa gtg   543
Leu Glu Gln Asn Leu Ser Asp Leu Gln Leu Gln Ala Ala Val Glu Val
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gcg ggg gac ctg gag gtc gat tgc tac cgg gca ccc tgc tcc gag agc   591
Ala Gly Asp Leu Glu Val Asp Cys Tyr Arg Ala Pro Cys Ser Glu Ser
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cag gag gag ctg gcc ctg cag cac ttc atg aag gag aag ctt ctg gct   639
Gln Glu Glu Leu Ala Leu Gln His Phe Met Lys Glu Lys Leu Leu Ala
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Glu Leu Glu Gly Lys Leu Arg Val Phe Glu Asn Ile Val Ala Val Leu
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aac aag gag gtg gag gcc tcc cac ctg gcc ctg gcc acc tct atc cac   735
Asn Lys Glu Val Glu Ala Ser His Leu Ala Leu Ala Thr Ser Ile His
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cag agc cag ctg gac cgt gag cgc atc ctg agc ttg gag cag agg gtg   783
Gln Ser Gln Leu Asp Arg Glu Arg Ile Leu Ser Leu Glu Gln Arg Val
                225                 230                 235

gtg gag ctt cag cag acc ctg gcc cag aaa gac cag gcc ctg ggc aag   831
Val Glu Leu Gln Gln Thr Leu Ala Gln Lys Asp Gln Ala Leu Gly Lys
            240                 245                 250

ctg gag cag agc ttg cgc ctc atg gag gag gcc tcc ttc gat ggc act   879
Leu Glu Gln Ser Leu Arg Leu Met Glu Glu Ala Ser Phe Asp Gly Thr
        255                 260                 265

ttc ctg tgg aag atc acc aat gtc acc agg cgg tgc cat gag tcg gcc   927
Phe Leu Trp Lys Ile Thr Asn Val Thr Arg Arg Cys His Glu Ser Ala
    270                 275                 280

tgt ggc agg acc gtc agc ctc ttc tcc cca gcc ttc tac act gcc aag   975
Cys Gly Arg Thr Val Ser Leu Phe Ser Pro Ala Phe Tyr Thr Ala Lys
285                 290                 295                 300

tat ggc tac aag ttg tgc ctg cgg ctg tac ctg aat gga gat ggc act  1023
Tyr Gly Tyr Lys Leu Cys Leu Arg Leu Tyr Leu Asn Gly Asp Gly Thr
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gga aag aga acc cat ctg tcg ctc ttc atc gtg atc atg aga ggg gag  1071
Gly Lys Arg Thr His Leu Ser Leu Phe Ile Val Ile Met Arg Gly Glu
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tat gat gcg ctg ctg ccg tgg ccc ttc cgg aac aag gtc acc ttc atg  1119
Tyr Asp Ala Leu Leu Pro Trp Pro Phe Arg Asn Lys Val Thr Phe Met
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ctg ctg gac cag aac aac cgt gag cac gcc att gac gcc ttc cgg cct  1167
Leu Leu Asp Gln Asn Asn Arg Glu His Ala Ile Asp Ala Phe Arg Pro
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gac cta agc tca gcg tcc ttc cag agg ccc cag agt gaa acc aac gtg   1215
Asp Leu Ser Ser Ala Ser Phe Gln Arg Pro Gln Ser Glu Thr Asn Val
365                 370                 375                 380

gcc agt gga tgc cca ctc ttc ttc ccc ctc agc aaa ctg cag tca ccc   1263
Ala Ser Gly Cys Pro Leu Phe Phe Pro Leu Ser Lys Leu Gln Ser Pro
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aag cac gcc tac gtg aag gac gac aca atg ttc ctc aag tgc att gtg   1311
Lys His Ala Tyr Val Lys Asp Asp Thr Met Phe Leu Lys Cys Ile Val
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gag acc agc act tag ggtgggcggg gctcctgagg gagctccaac tcagaaggga   1366
Glu Thr Ser Thr
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Ala Ala Ala Ser Val Thr Pro Pro Gly Ser Leu Glu Leu Leu Gln Pro
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Gly Phe Ser Lys Thr Leu Leu Gly Thr Lys Leu Glu Ala Lys Tyr Leu
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tgc tcc gcc tgc aga aac gtc ctc cgc agg ccc ttc cag gcg cag tgt   201
Cys Ser Ala Cys Arg Asn Val Leu Arg Arg Pro Phe Gln Ala Gln Cys
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ggc cac cgg tac tgc tcc ttc tgc ctg gcc agc atc ctc agc tct ggg   249
Gly His Arg Tyr Cys Ser Phe Cys Leu Ala Ser Ile Leu Ser Ser Gly
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cct cag aac tgt gct gcc tgt gtt cac gag ggc ata tat gaa gaa ggc   297
Pro Gln Asn Cys Ala Ala Cys Val His Glu Gly Ile Tyr Glu Glu Gly
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att tct att tta gaa agc agt tcg gcc ttc cca gat aat gct gcc cgc   345
Ile Ser Ile Leu Glu Ser Ser Ser Ala Phe Pro Asp Asn Ala Ala Arg
             85                  90                  95

agg gag gtg gag agc ctg ccg gcc gtc tgt ccc agt gat gga tgc acc   393
Arg Glu Val Glu Ser Leu Pro Ala Val Cys Pro Ser Asp Gly Cys Thr
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tgg aag ggg acc ctg aaa gaa tac gag agc tgc cac gaa ggc cgc tgc   441
Trp Lys Gly Thr Leu Lys Glu Tyr Glu Ser Cys His Glu Gly Arg Cys
     115                 120                 125

ccg ctc atg ctg acc gaa tgt ccc gcg tgt aaa ggc ctg gtc cgc ctt   489
Pro Leu Met Leu Thr Glu Cys Pro Ala Cys Lys Gly Leu Val Arg Leu
130                 135                 140                 145

ggt gaa aag gag cgc cac ctg gag cac gag tgc ccg gag aga agc ctg   537
Gly Glu Lys Glu Arg His Leu Glu His Glu Cys Pro Glu Arg Ser Leu
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agc tgc cgg cat tgc cgg gca ccc tgc tgc gga gca gac gtg aag gcg   585
Ser Cys Arg His Cys Arg Ala Pro Cys Cys Gly Ala Asp Val Lys Ala
            165                 170                 175

cac cac gag gtc tgc ccc aag ttc ccc tta act tgt gac ggc tgc ggc   633
His His Glu Val Cys Pro Lys Phe Pro Leu Thr Cys Asp Gly Cys Gly
        180                 185                 190

aag aag aag atc ccc cgg gag aag ttt cag gac cac gtc aag act tgt   681
Lys Lys Lys Ile Pro Arg Glu Lys Phe Gln Asp His Val Lys Thr Cys
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ggc aag tgt cga gtc cct tgc aga ttc cac gcc atc ggc tgc ctc gag   729
Gly Lys Cys Arg Val Pro Cys Arg Phe His Ala Ile Gly Cys Leu Glu
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acg gta gag ggt gag aaa cag cag gag cac gag gtg cag tgg ctg cgg   777
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Thr Val Glu Gly Glu Lys Gln Gln Glu His Glu Val Gln Trp Leu Arg
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gag cac ctg gcc atg cta ctg agc tcg gtg ctg gag gca aag ccc ctc   825
Glu His Leu Ala Met Leu Leu Ser Ser Val Leu Glu Ala Lys Pro Leu
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ttg gga gac cag agc cac gcg ggg tca gag ctc ctg cag agg tgc gag   873
Leu Gly Asp Gln Ser His Ala Gly Ser Glu Leu Leu Gln Arg Cys Glu
        260                 265                 270

agc ctg gag aag aag acg gcc act ttt gag aac att gtc tgc gtc ctg   921
Ser Leu Glu Lys Lys Thr Ala Thr Phe Glu Asn Ile Val Cys Val Leu
    275                 280                 285

aac cgg gag gtg gag agg gtg gcc atg act gcc gag gcc tgc agc cgg   969
Asn Arg Glu Val Glu Arg Val Ala Met Thr Ala Glu Ala Cys Ser Arg
290                 295                 300                 305

cag cac cgg ctg gac caa gac aag att gaa gcc ctg agt agc aag gtg   1017
Gln His Arg Leu Asp Gln Asp Lys Ile Glu Ala Leu Ser Ser Lys Val
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cag cag ctg gag agg agc att ggc ctc aag gac ctg gcg atg gct gac   1065
Gln Gln Leu Glu Arg Ser Ile Gly Leu Lys Asp Leu Ala Met Ala Asp
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ttg gag cag aag gtc agg ccc ttc cag gcg cag tgt ggc cac cgg tac   1113
Leu Glu Gln Lys Val Arg Pro Phe Gln Ala Gln Cys Gly His Arg Tyr
        340                 345                 350

tgc tcc ttc tgc ctg gcc agc atc ctc agg aag ctc cag gaa gct gtg   1161
Cys Ser Phe Cys Leu Ala Ser Ile Leu Arg Lys Leu Gln Glu Ala Val
    355                 360                 365

gct ggc cgc ata ccc gcc atc ttc tcc cca gcc ttc tac acc agc agg   1209
Ala Gly Arg Ile Pro Ala Ile Phe Ser Pro Ala Phe Tyr Thr Ser Arg
370                 375                 380                 385

tac ggc tac aag atg tgt ctg cgt atc tac ctg aac ggc gac ggc acc   1257
Tyr Gly Tyr Lys Met Cys Leu Arg Ile Tyr Leu Asn Gly Asp Gly Thr
                390                 395                 400

ggg cga gga aca cac ctg tcc ctc ttc ttt gtg gtg atg aag ggc ccg   1305
Gly Arg Gly Thr His Leu Ser Leu Phe Phe Val Val Met Lys Gly Pro
            405                 410                 415

aat gac gcc ctg ctg cgg tgg ccc ttc aac cag aag gtg acc tta atg   1353
Asn Asp Ala Leu Leu Arg Trp Pro Phe Asn Gln Lys Val Thr Leu Met
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ctg ctc gac cag aat aac cgg gag cac gtg att gac gcc ttc agg ccc   1401
Leu Leu Asp Gln Asn Asn Arg Glu His Val Ile Asp Ala Phe Arg Pro
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gac gtg act tca tcc tct ttt cag agg cca gtc aac gac atg aac atc   1449
Asp Val Thr Ser Ser Ser Phe Gln Arg Pro Val Asn Asp Met Asn Ile
450                 455                 460                 465

gca agc ggc tgc ccc ctc ttc tgc ccc gtc tcc aag atg gag gca aag   1497
Ala Ser Gly Cys Pro Leu Phe Cys Pro Val Ser Lys Met Glu Ala Lys
                470                 475                 480
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aat tcc tac gtg cgg gac gat gcc atc ttc atc aag gcc att gtg gac   1545
Asn Ser Tyr Val Arg Asp Asp Ala Ile Phe Ile Lys Ala Ile Val Asp
            485                 490                 495

ctg aca ggg ctc taa ctgcccccta ctggtgtctg ggggttgggg gcagccaggc   1600
Leu Thr Gly Leu
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gagcggcgac ggaccgcgag aactcctctt tcctaaa atg gag tcg agt aaa aag  235
                                        Met Glu Ser Ser Lys Lys
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atg gac tct cct ggc gcg ctg cag act aac ccg ccg cta aag ctg cac   283
Met Asp Ser Pro Gly Ala Leu Gln Thr Asn Pro Pro Leu Lys Leu His
            10                  15                  20

act gac cgt agt gct ggg acg cca gtt ttt gtc cct gaa caa gga ggt   331
Thr Asp Arg Ser Ala Gly Thr Pro Val Phe Val Pro Glu Gln Gly Gly
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tac aag gaa aag ttt gtg aag acc gtg gag gac aag tac aag tgt gag   379
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aag tgc cac ctg gtg ctg tgc agc ccg aag cag acc gag tgt ggg cac   427
Lys Cys His Leu Val Leu Cys Ser Pro Lys Gln Thr Glu Cys Gly His
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cgc ttc tgc gag agc tgc atg gcg gcc ctg ctg agc tct tca agt cca   475
Arg Phe Cys Glu Ser Cys Met Ala Ala Leu Leu Ser Ser Ser Ser Pro
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aaa tgt aca gcg tgt caa gag agc atc gtt aaa gat aag gtg ttt aag   523
Lys Cys Thr Ala Cys Gln Glu Ser Ile Val Lys Asp Lys Val Phe Lys
             90                  95                 100

gat aat tgc tgc aag aga gaa att ctg gct ctt cag atc tat tgt cgg   571
Asp Asn Cys Cys Lys Arg Glu Ile Leu Ala Leu Gln Ile Tyr Cys Arg
        105                 110                 115

aat gaa agc aga ggt tgt gca gag cag tta acg ctg gga cat ctg ctg   619
Asn Glu Ser Arg Gly Cys Ala Glu Gln Leu Thr Leu Gly His Leu Leu
    120                 125                 130

gtg cat tta aaa aat gat tgc cat ttt gaa gaa ctt cca tgt gtg cgt   667
Val His Leu Lys Asn Asp Cys His Phe Glu Glu Leu Pro Cys Val Arg
135                 140                 145                 150

cct gac tgc aaa gaa aag gtc ttg agg aaa gac ctg cga gac cac gtg   715
Pro Asp Cys Lys Glu Lys Val Leu Arg Lys Asp Leu Arg Asp His Val
                155                 160                 165

gag aag gcg tgt aaa tac cgg gaa gcc aca tgc agc cac tgc aag agt   763
Glu Lys Ala Cys Lys Tyr Arg Glu Ala Thr Cys Ser His Cys Lys Ser
            170                 175                 180

cag gtt ccg atg atc gcg ctg cag aaa cac gaa gac acc gac tgt ccc   811
Gln Val Pro Met Ile Ala Leu Gln Lys His Glu Asp Thr Asp Cys Pro
        185                 190                 195

tgc gtg gtg gtc tcc tgc cct cac aag tgc agc gtc cag act ctc ctg   859
Cys Val Val Val Ser Cys Pro His Lys Cys Ser Val Gln Thr Leu Leu
    200                 205                 210

agg agc gag ttg agt gca cac ttg tca gag tgt gtc aat gcc ccc agc   907
Arg Ser Glu Leu Ser Ala His Leu Ser Glu Cys Val Asn Ala Pro Ser
215                 220                 225                 230

acc tgt agt ttt aag cgc tat ggc tgc gtt ttt cag ggg aca aac cag   955
Thr Cys Ser Phe Lys Arg Tyr Gly Cys Val Phe Gln Gly Thr Asn Gln
                235                 240                 245

cag atc aag gcc cac gag gcc agc tcc gcc gtg cag cac gtc aac ctg  1003
Gln Ile Lys Ala His Glu Ala Ser Ser Ala Val Gln His Val Asn Leu
            250                 255                 260

ctg aag gag tgg agc aac tcg ctc gaa aag aag gtt tcc ttg ttg cag  1051
Leu Lys Glu Trp Ser Asn Ser Leu Glu Lys Lys Val Ser Leu Leu Gln
        265                 270                 275

aat gaa agt gta gaa aaa aac aag agc ata caa agt ttg cac aat cag  1099
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Ile Cys Ser Phe Glu Ile Glu Ile Glu Arg Gln Lys Glu Met Leu Arg
295                 300                 305                 310

aat aat gaa tcc aaa atc ctt cat tta cag cga gtg atc gac agc caa   1195
Asn Asn Glu Ser Lys Ile Leu His Leu Gln Arg Val Ile Asp Ser Gln
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gca gag aaa ctg aag gag ctt gac aag gag atc cgg ccc ttc cgg cag   1243
Ala Glu Lys Leu Lys Glu Leu Asp Lys Glu Ile Arg Pro Phe Arg Gln
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aac tgg gag gaa gca gac agc atg aag agc agc gtg gag tcc ctc cag   1291
Asn Trp Glu Glu Ala Asp Ser Met Lys Ser Ser Val Glu Ser Leu Gln
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aac cgc gtg acc gag ctg gag agc gtg gac aag agt gcg ggg caa gtg   1339
Asn Arg Val Thr Glu Leu Glu Ser Val Asp Lys Ser Ala Gly Gln Val
    360                 365                 370

gct cgg aac aca ggc ctg ctg gag tcc cag ctg agc cgg cat gac cag   1387
Ala Arg Asn Thr Gly Leu Leu Glu Ser Gln Leu Ser Arg His Asp Gln
375                 380                 385                 390

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cag gtc ctg gag acc gcc agc tac aat gga gtg ctc atc tgg aag att   1483
Gln Val Leu Glu Thr Ala Ser Tyr Asn Gly Val Leu Ile Trp Lys Ile
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Arg Asp Tyr Lys Arg Arg Lys Gln Glu Ala Val Met Gly Lys Thr Leu
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Ser Leu Tyr Ser Gln Pro Phe Tyr Thr Gly Tyr Phe Gly Tyr Lys Met
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tgt gcc agg gtc tac ctg aac ggg gac ggg atg ggg aag ggg acg cac   1627
Cys Ala Arg Val Tyr Leu Asn Gly Asp Gly Met Gly Lys Gly Thr His
455                 460                 465                 470

ttg tcg ctg ttt ttt gtc atc atg cgt gga gaa tat gat gcc ctg ctt   1675
Leu Ser Leu Phe Phe Val Ile Met Arg Gly Glu Tyr Asp Ala Leu Leu
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cct tgg ccg ttt aag cag aaa gtg aca ctc atg ctg atg gat cag ggg   1723
Pro Trp Pro Phe Lys Gln Lys Val Thr Leu Met Leu Met Asp Gln Gly
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tcc tct cga cgt cat ttg gga gat gca ttc aag ccc gac ccc aac agc   1771
Ser Ser Arg Arg His Leu Gly Asp Ala Phe Lys Pro Asp Pro Asn Ser
        505                 510                 515

agc agc ttc aag aag ccc act gga gag atg aat atc gcc tct ggc tgc   1819
Ser Ser Phe Lys Lys Pro Thr Gly Glu Met Asn Ile Ala Ser Gly Cys
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cca gtc ttt gtg gcc caa act gtt cta gaa aat ggg aca tat att aaa   1867
Pro Val Phe Val Ala Gln Thr Val Leu Glu Asn Gly Thr Tyr Ile Lys
535                 540                 545                 550

gat gat aca att ttt att aaa gtc ata gtg gat act tcg gat ctg ccc   1915
Asp Asp Thr Ile Phe Ile Lys Val Ile Val Asp Thr Ser Asp Leu Pro
                555                 560                 565

gat ccc tga taagtagctg gggaggtgga tttagcagaa ggcaactcct           1964
Asp Pro

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gag aag ccc aag cga cgg ctg ctg tgc cca ctg tgc ggg aag ccc atg   160
Glu Lys Pro Lys Arg Arg Leu Leu Cys Pro Leu Cys Gly Lys Pro Met
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Arg Glu Pro Val Gln Val Ser Thr Cys Gly His Arg Phe Cys Asp Thr
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tgc ctg cag gag ttc ctc agt gaa gga gtc ttc aag tgc cct gag gac   256
Cys Leu Gln Glu Phe Leu Ser Glu Gly Val Phe Lys Cys Pro Glu Asp
             45                  50                  55

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Val Gln Val Leu Gly Leu Pro Ile Arg Cys Ile His Ser Glu Glu Gly
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tgc cgc tgg agt ggg cca cta cgt cat cta cag ggc cac ctg aat acc   400
Cys Arg Trp Ser Gly Pro Leu Arg His Leu Gln Gly His Leu Asn Thr
 90                  95                 100                 105

tgc agc ttc aat gtc att ccc tgc cct aat cgc tgc ccc atg aag ctg   448
Cys Ser Phe Asn Val Ile Pro Cys Pro Asn Arg Cys Pro Met Lys Leu
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agc cgc cgt gat cta cct gca cac ttg cag cat gac tgc ccc aag cgg   496
Ser Arg Arg Asp Leu Pro Ala His Leu Gln His Asp Cys Pro Lys Arg
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cgc ctc aag tgc gag ttt tgt ggc tgt gac ttc agt ggg gag gcc tat   544
Arg Leu Lys Cys Glu Phe Cys Gly Cys Asp Phe Ser Gly Glu Ala Tyr
        140                 145                 150

gag agc cat gag ggt atg tgc ccc cag gag agt gtc tac tgt gag aat   592
Glu Ser His Glu Gly Met Cys Pro Gln Glu Ser Val Tyr Cys Glu Asn
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aag tgt ggt gcc cgc atg atg cgg ggg ctg ctg gcc cag cat gcc acc   640
Lys Cys Gly Ala Arg Met Met Arg Gly Leu Leu Ala Gln His Ala Thr
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tct gag tgc ccc aag cgc act cag ccc tgc acc tac tgc act aag gag   688
Ser Glu Cys Pro Lys Arg Thr Gln Pro Cys Thr Tyr Cys Thr Lys Glu
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Phe Val Phe Asp Thr Ile Gln Ser His Gln Tyr Gln Cys Pro Arg Leu
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cct gtt gcc tgc ccc aac caa tgt ggt gtg ggc act gtg gct cgg gag   784
Pro Val Ala Cys Pro Asn Gln Cys Gly Val Gly Thr Val Ala Arg Glu
        220                 225                 230

gac ctg cca ggc cat ctg aag gac agc tgt aac acc gcc ctg gtg ctc   832
Asp Leu Pro Gly His Leu Lys Asp Ser Cys Asn Thr Ala Leu Val Leu
    235                 240                 245

tgc cca ttc aaa gac tcc ggc tgc aag cac cgg tgc cct aag ctg gca   880
Cys Pro Phe Lys Asp Ser Gly Cys Lys His Arg Cys Pro Lys Leu Ala
250                 255                 260                 265

atg gca cgg cat gtg gag gag agt gtg aag cca cat ctg gcc atg atg   928
Met Ala Arg His Val Glu Glu Ser Val Lys Pro His Leu Ala Met Met
                270                 275                 280

tgt gcc ctg gtg agc cgg caa cgg cag gag ctg cag gag ctt cgg cga   976
Cys Ala Leu Val Ser Arg Gln Arg Gln Glu Leu Gln Glu Leu Arg Arg
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gag ctg gag gag cta tca gtg ggc agt gat ggc gtg ctc atc tgg aag  1024
Glu Leu Glu Glu Leu Ser Val Gly Ser Asp Gly Val Leu Ile Trp Lys
        300                 305                 310
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Ile Gly Ser Tyr Gly Arg Arg Leu Gln Glu Ala Lys Ala Lys Pro Asn
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ctt gag tgc ttc agc cca gcc ttc tac aca cat aag tat ggt tac aag   1120
Leu Glu Cys Phe Ser Pro Ala Phe Tyr Thr His Lys Tyr Gly Tyr Lys
330                 335                 340                 345

ctg cag gtg tct gca ttc ctc aat ggc aat ggc agt ggt gag ggc aca   1168
Leu Gln Val Ser Ala Phe Leu Asn Gly Asn Gly Ser Gly Glu Gly Thr
                350                 355                 360

cac ctc tca ctg tac att cgt gtg ctg cct ggt gcc ttt gac aat ctc   1216
His Leu Ser Leu Tyr Ile Arg Val Leu Pro Gly Ala Phe Asp Asn Leu
            365                 370                 375

ctt gag tgg ccc ttt gcc cgc cgt gtc acc ttc tcc ctg ctg gat cag   1264
Leu Glu Trp Pro Phe Ala Arg Arg Val Thr Phe Ser Leu Leu Asp Gln
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agc gac cct ggg ctg gct aaa cca cag cac gtc act gag acc ttc cac   1312
Ser Asp Pro Gly Leu Ala Lys Pro Gln His Val Thr Glu Thr Phe His
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ccc gac cca aac tgg aag aat ttc cag aag cca ggc acg tgg cgg ggc   1360
Pro Asp Pro Asn Trp Lys Asn Phe Gln Lys Pro Gly Thr Trp Arg Gly
410                 415                 420                 425

tcc ctg gat gag agt tct ctg ggc ttt ggt tat ccc aag ttc atc tcc   1408
Ser Leu Asp Glu Ser Ser Leu Gly Phe Gly Tyr Pro Lys Phe Ile Ser
                430                 435                 440

cac cag gac att cga aag cga aac tat gtg cgg gat gat gca gtc ttc   1456
His Gln Asp Ile Arg Lys Arg Asn Tyr Val Arg Asp Asp Ala Val Phe
            445                 450                 455

atc cgt gct gct gtt gaa ctg ccc cgg aag atc ctc agc tga           1498
Ile Arg Ala Ala Val Glu Leu Pro Arg Lys Ile Leu Ser
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Ala Tyr Ser Glu Glu His Lys Gly Met Pro Cys Gly Phe Ile Arg Gln
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aat tcc ggc aac tcc att tcc ttg gac ttt gag ccc agt ata gag tac   153
Asn Ser Gly Asn Ser Ile Ser Leu Asp Phe Glu Pro Ser Ile Glu Tyr
          20                  25                  30

cag ttt gtg gag cgg ctg gaa gag cgc tac aaa tgt gcc ttc tgc cac   201
Gln Phe Val Glu Arg Leu Glu Glu Arg Tyr Lys Cys Ala Phe Cys His
      35                  40                  45

tcg gtg ctt cac aac ccc cac cag aca gga tgt ggg cac cgc ttc tgc   249
Ser Val Leu His Asn Pro His Gln Thr Gly Cys Gly His Arg Phe Cys
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cag cac tgc atc ctg tcc ctg aga gaa tta aac aca gtg cca atc tgc   297
Gln His Cys Ile Leu Ser Leu Arg Glu Leu Asn Thr Val Pro Ile Cys
                 70                  75                  80

cct gta gat aaa gag gtc atc aaa tct cag gag gtt ttt aaa gac aat   345
Pro Val Asp Lys Glu Val Ile Lys Ser Gln Glu Val Phe Lys Asp Asn
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tgt tgc aaa aga gaa gtc ctc aac tta tat gta tat tgc agc aat gct   393
Cys Cys Lys Arg Glu Val Leu Asn Leu Tyr Val Tyr Cys Ser Asn Ala
          100                 105                 110

cct gga tgt aat gcc aag gtt att ctg ggc cgg tac cag gat cac ctt   441
Pro Gly Cys Asn Ala Lys Val Ile Leu Gly Arg Tyr Gln Asp His Leu
      115                 120                 125

cag cag tgc tta ttt caa cct gtg cag tgt tct aat gag aag tgc cgg   489
Gln Gln Cys Leu Phe Gln Pro Val Gln Cys Ser Asn Glu Lys Cys Arg
130                 135                 140                 145

gag cca gtc cta cgg aaa gac ctg aaa gag cat ttg agt gca tcc tgt   537
Glu Pro Val Leu Arg Lys Asp Leu Lys Glu His Leu Ser Ala Ser Cys
                150                 155                 160

cag ttt cga aag gaa aaa tgc ctt tat tgc aaa aag gat gtg gta gtc   585
Gln Phe Arg Lys Glu Lys Cys Leu Tyr Cys Lys Lys Asp Val Val Val
              165                 170                 175

atc aat cta cag aat cat gag gaa aac ttg tgt cct gaa tac cca gta   633
Ile Asn Leu Gln Asn His Glu Glu Asn Leu Cys Pro Glu Tyr Pro Val
          180                 185                 190

ttt tgt ccc aac aat tgt gcg aag att att cta aaa act gag gta gat   681
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Phe Cys Pro Asn Asn Cys Ala Lys Ile Ile Leu Lys Thr Glu Val Asp
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gaa cac ctg gct gta tgt cct gaa gct gag caa gac tgt cct ttt aag   729
Glu His Leu Ala Val Cys Pro Glu Ala Glu Gln Asp Cys Pro Phe Lys
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cac tat ggc tgt gct gta acg gat aaa cgg agg aac ctg cag caa cat   777
His Tyr Gly Cys Ala Val Thr Asp Lys Arg Arg Asn Leu Gln Gln His
                230                 235                 240

gag cat tca gcc tta cgg gag cac atg cgt ttg gtt tta gaa aag aat   825
Glu His Ser Ala Leu Arg Glu His Met Arg Leu Val Leu Glu Lys Asn
            245                 250                 255

gtc caa tta gaa gaa cag att tct gac tta cac aag agc cta gaa cag   873
Val Gln Leu Glu Glu Gln Ile Ser Asp Leu His Lys Ser Leu Glu Gln
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aaa gaa agt aaa atc cag cag cta gca gaa act ata aag aaa ctt gaa   921
Lys Glu Ser Lys Ile Gln Gln Leu Ala Glu Thr Ile Lys Lys Leu Glu
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aag gag ttc aag cag ttt gca cag ttg ttt ggc aaa aat gga agc ttc   969
Lys Glu Phe Lys Gln Phe Ala Gln Leu Phe Gly Lys Asn Gly Ser Phe
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ctc cca aac atc cag gtt ttt gcc agt cac att gac aag tca gct tgg  1017
Leu Pro Asn Ile Gln Val Phe Ala Ser His Ile Asp Lys Ser Ala Trp
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cta gaa gct caa gtg cat caa tta tta caa atg gtt aac cag caa caa  1065
Leu Glu Ala Gln Val His Gln Leu Leu Gln Met Val Asn Gln Gln Gln
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aat aaa ttt gac ctg aga cct ttg atg gaa gca gtt gat aca gtg aaa  1113
Asn Lys Phe Asp Leu Arg Pro Leu Met Glu Ala Val Asp Thr Val Lys
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cag aaa att acc ctg cta gaa aac aat gat caa aga tta gcc gtt tta  1161
Gln Lys Ile Thr Leu Leu Glu Asn Asn Asp Gln Arg Leu Ala Val Leu
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gaa gag gaa act aac aaa cat gat acc cac att aat att cat aaa gca  1209
Glu Glu Glu Thr Asn Lys His Asp Thr His Ile Asn Ile His Lys Ala
370                 375                 380                 385

cag ctg agt aaa aat gaa gag cga ttt aaa ctg ctg gag ggt act tgc  1257
Gln Leu Ser Lys Asn Glu Glu Arg Phe Lys Leu Leu Glu Gly Thr Cys
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tat aat gga aag ctc att tgg aag gtg aca gat tac aag atg aag aag  1305
Tyr Asn Gly Lys Leu Ile Trp Lys Val Thr Asp Tyr Lys Met Lys Lys
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aga gag gcg gtg gat ggg cac aca gtg tcc atc ttc agc cag tcc ttc  1353
Arg Glu Ala Val Asp Gly His Thr Val Ser Ile Phe Ser Gln Ser Phe
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tac acc agc cgc tgt ggc tac cgg ctc tgt gct aga gca tac ctg aat  1401
Tyr Thr Ser Arg Cys Gly Tyr Arg Leu Cys Ala Arg Ala Tyr Leu Asn
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Gly Asp Gly Ser Gly Arg Gly Ser His Leu Ser Leu Tyr Phe Val Val
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atg cga gga gag ttt gac tca ctg ttg cag tgg cca ttc agg cag agg   1497
Met Arg Gly Glu Phe Asp Ser Leu Leu Gln Trp Pro Phe Arg Gln Arg
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gtg acc ctg atg ctt ctg gac cag agt ggc aaa aag aac att atg gag   1545
Val Thr Leu Met Leu Leu Asp Gln Ser Gly Lys Lys Asn Ile Met Glu
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acc ttc aaa cct gac ccc aat agc agc agc ttt aaa aga cct gat ggg   1593
Thr Phe Lys Pro Asp Pro Asn Ser Ser Ser Phe Lys Arg Pro Asp Gly
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gag atg aac att gca tct ggc tgt ccc cgc ttt gtg gct cat tct gtt   1641
Glu Met Asn Ile Ala Ser Gly Cys Pro Arg Phe Val Ala His Ser Val
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Leu Glu Asn Ala Lys Asn Ala Tyr Ile Lys Asp Asp Thr Leu Phe Leu
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aaa gtg gcc gtg gac tta act gac ctg gag gat ctc tag tcactgttat    1738
Lys Val Ala Val Asp Leu Thr Asp Leu Glu Asp Leu
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Phe Glu Asp Lys Glu Ile His Asp Gln Asn Cys Pro Leu Ala Asn Val
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atc tgt gaa tac tgc aat act ata ctc atc aga gaa cag atg cct aat   908
Ile Cys Glu Tyr Cys Asn Thr Ile Leu Ile Arg Glu Gln Met Pro Asn
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cat tat gat cta gac tgc cct aca gcc cca att cca tgc aca ttc agt   956
His Tyr Asp Leu Asp Cys Pro Thr Ala Pro Ile Pro Cys Thr Phe Ser
230                 235                 240                 245

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cta caa gag aac acc cag tca cac atg aga atg ttg gcc cag gct gtt   1052
Leu Gln Glu Asn Thr Gln Ser His Met Arg Met Leu Ala Gln Ala Val
            265                 270                 275

cat agt ttg agc gtt ata ccc gac tct ggg tat atc tca gag gtc cgg   1100
His Ser Leu Ser Val Ile Pro Asp Ser Gly Tyr Ile Ser Glu Val Arg
        280                 285                 290

aat ttc cag gaa act att cac cag tta gag ggt cgc ctt gta aga caa   1148
Asn Phe Gln Glu Thr Ile His Gln Leu Glu Gly Arg Leu Val Arg Gln
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gac cat caa atc cgg gag ctg act gct aaa atg gaa act cag agt atg   1196
Asp His Gln Ile Arg Glu Leu Thr Ala Lys Met Glu Thr Gln Ser Met
310                 315                 320                 325

tat gta agt gag ctc aaa cga acc att cga acc ctt gag gac aaa gtt   1244
Tyr Val Ser Glu Leu Lys Arg Thr Ile Arg Thr Leu Glu Asp Lys Val
                330                 335                 340

gct gaa atc gaa gca cag cag tgc aat gga att tat att tgg aag att   1292
Ala Glu Ile Glu Ala Gln Gln Cys Asn Gly Ile Tyr Ile Trp Lys Ile
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Gly Asn Phe Gly Met His Leu Lys Cys Gln Glu Glu Glu Lys Pro Val
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Val Ile His Ser Pro Gly Phe Tyr Thr Gly Lys Pro Gly Tyr Lys Leu
    375                 380                 385

tgc atg cgc ttg cac ctt cag tta ccg act gct cag cgc tgt gca aac   1436
Cys Met Arg Leu His Leu Gln Leu Pro Thr Ala Gln Arg Cys Ala Asn
390                 395                 400                 405

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Tyr Ile Ser Leu Phe Val His Thr Met Gln Gly Glu Tyr Asp Ser His
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Leu Pro Trp Pro Phe Gln Gly Thr Ile Arg Leu Thr Ile Leu Asp Gln
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tct gaa gca cct gta agg caa aac cac gaa gag ata atg gat gcc aaa   1580
Ser Glu Ala Pro Val Arg Gln Asn His Glu Glu Ile Met Asp Ala Lys
        440                 445                 450

cca gag ctg ctt gct ttc cag cga ccc aca atc cca cgg aac cca aaa   1628
Pro Glu Leu Leu Ala Phe Gln Arg Pro Thr Ile Pro Arg Asn Pro Lys
    455                 460                 465

ggt ttt ggc tat gta act ttt atg cat ctg gaa gcc cta aga caa aga   1676
Gly Phe Gly Tyr Val Thr Phe Met His Leu Glu Ala Leu Arg Gln Arg
470                 475                 480                 485

act ttc att aag gat gac aca tta tta gtg cgc tgt gag gtc tcc acc   1724
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23171

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : C07H 21/04; C12Q 1/68; A61K 48/00
US CL : 435/6; 514/44; 536/23.1, 24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 514/44; 536/23.1, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, SCISEARCH, CAPLUS, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	XU et al. A Role for TRAF 2 and TRAF 6 in Cytokine-Mediated Induction of E-selectin. Immunity. 1996. vol. 5, pages 407-415, see entire document.	1-7, 9-14, and 17-19 --- 8
Y	SANGHVI et al. HETEROCYCLIC BASE MODIFICATIONS IN NUECLEIC ACIDS AND THEIR APPLICATIONS IN ANTISENSE OLIGONUCLEOTIDES. Antisense Research and Applications, pages 273-288, see entire document.	8
A	BRANCH, A good antisense molecule is hard to find. TIBS, February 1998. vol. 23, pages 45-50, see entire document.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 NOVEMBER 1999	Date of mailing of the international search report 04 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ANDREW WANG Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*